VOLUME 46 FEBRUARY 1960 NUMBER 1

The Journal of PARASITOLOGY

FOUNDED BY HENRY BALDWIN WARD

OFFICIAL ORGAN OF THE
AMERICAN SOCIETY OF PARASITOLOGISTS

EDITOR
ELERY R. BECKER

ASSOCIATE EDITORS
HAROLD W. MANTER
HERBERT T. DALMAT

ASSISTANT EDITORS

RAYMOND M. CABLE
QUENTIN M. GEIMAN
DOMINIC L. DeGIUSTI
CHAUNCEY G. GOODCHILD
GLEN M. KOHLS
GEORGE R. LA RUE
ROBERT M. LEWERT
R. BARCLAY McCHEE
ALLEN McINTOSH
CLARK P. READ
RALPH E. THORSON
PAUL P. WEINSTEIN

AMERICAN SOCIETY OF PARASITOLOGISTS

OFFICERS AND COUNCIL MEMBERS

President: HAROLD W. BROWN
Columbia University
School of Public Health
New York 32, New York

President-Elect: JUSTIN M. ANDREWS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda 14, Maryland

Vice-President: PAUL E. THOMPSON
Research Division
Parke, Davis and Company
Detroit 32, Michigan

Secretary: FRANCIS J. KRUIDENIER
Dept. of Zoology and Physiology
University of Illinois
Urbana, Illinois

Treasurer: MARTIN J. ULMER
Dept. of Zoology and Entomology
Iowa State University
Ames, Iowa

Custodian: WILFORD S. BAILEY
School of Veterinary Medicine
Alabama Polytechnic Institute
Auburn, Alabama

Editor: ELERY R. BECKER
Life Science Center
Arizona State University
Tempe, Arizona

Council Members-at-Large: Paul P. Weinstein, Donald V. Moore, Robert M. Lewert, Clark P. Read, Ralph V. Bangham, Louis J. Olivier, Gordon H. Ball, Dale A. Porter. Non-Voting Council Representatives: Southern California Parasitologists, Marietta Voge; Annual Midwestern Conference of Parasitologists, David T. Clark.

Persons interested in membership in the American Society of Parasitologists may obtain application blanks from the Secretary. A year's subscription to the Journal of Parasitology is included in the annual dues ($8.00).

THE JOURNAL OF PARASITOLOGY

The Journal of Parasitology, published bi-monthly, is both the official organ of the American Society of Parasitologists and a medium for the publication of the results of research in the field of animal parasitology.

Manuscripts intended for publication may be sent to the Editor or any other member of the Editorial Board with the understanding that they are to be published exclusively in the Journal unless otherwise arranged in advance. All papers received will be submitted to competent reviewers, either members of the Board or other persons with special qualifications. Authors will be notified when their papers have been accepted for publication. Correspondence regarding manuscripts should be addressed to the Editor (address above).

Manuscripts should be typewritten, double-spaced, with wide margins at sides, top and bottom, and with tables and illustrations in finished form. Sentences should be concise and clear, and the text should be as condensed as possible because of mounting costs of publication. Title, author, address, etc., as well as footnotes, should appear on a separate sheet. Footnotes should be avoided whenever possible. Each table requires a separate sheet. Tables should be simple, since complex ones are difficult to inspect.

Tabulation should be avoided when the material can as well be summarized in the text. Extensive duplication or triplication of data in text, tables and graphs should also be avoided. Sharp, contrasty, glossy photographs of plates that will fit into a 9- × 12-inch envelope should be sent instead of the originals, since the latter have often been badly damaged in the mails. Figures should be arranged within a 5- × 8-inch area or multiple thereof. Legends for figures should be typed on a page separate from the text and illustrations. Greek characters and symbols should be avoided on account of cost.

Bibliography should include only references cited in the text, and its style should conform to that in recent issues of the Journal. The abbreviations for names of journals employed by the Index Catalog of Medical and Veterinary Zoology are recommended to authors.

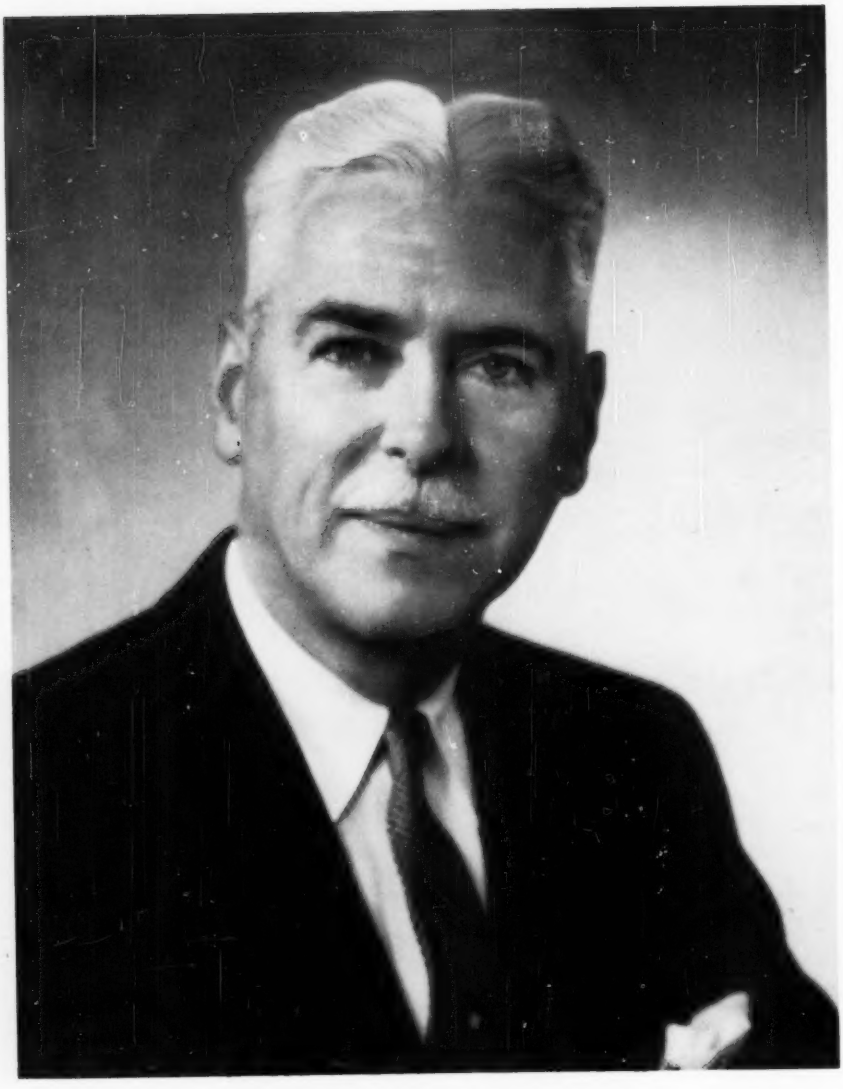
Acceptability of a paper, however, is determined not by its length, but by the amount and character of new information included and the form in which it is presented. Long articles and those with an unusually large number of plates can be published only with the financial cooperation of the author.

Reprints may be purchased at cost from the printer if they are ordered when the galley proof is returned. Claims for copies lost in the mails must be received by the Treasurer within 60 days of the mailing date. Delivery overseas must be at the subscriber's risk. Change of address must be received by the Treasurer 2 weeks prior to mailing date.

The subscription price for the Journal is $9.50 domestic, $10.25 foreign. Orders should be addressed to the Treasurer (address above).

Correspondence concerning back issues should be addressed to the Custodian (address above).

Entered as second-class matter December 21, 1932, at the post office at Lancaster, Pa., under the Act of March 3, 1879.



Arnold O. Foster

The Journal of Parasitology

Volume 46 FEBRUARY, 1960 Number 1

PARASITOLOGICAL SPECULATIONS AND PATTERNS*

AUREL O. FOSTER

Animal Disease and Parasite Research Division,
Agricultural Research Service, U. S. Department of Agriculture,
Beltsville, Maryland

When there was reason to suspect the inevitability of this occasion, there seemed also reason to suspect that we should be remiss if these moments were not devoted to practical considerations of our Society and discipline. There is much that ought seemingly be said openly and which we ought freely to discuss.

One who senses the pulse of this group cannot but feel that our Society, even as our discipline, is destined increasingly to achieve genuine worth, and the more so through objective review and self-criticism. Perhaps the significant aspect of the splendid cooperation, which it has been my unforgettable experience to know and enjoy, is its homely and incontrovertible proof that our Society and discipline are secure under the sharpest scrutiny.

A most heartening experience of the year was the realization that every one of some seventy of our busy members whom it was necessary to call upon to accept an assignment of responsibility and service proved ready and willing not only to do what was asked of him, but also to share and unstintingly work toward the common objective of positive, step-by-step action in the direction of what seemed truly best for parasitology, parasitologists, and the American Society of Parasitologists; and best also for all persons and interests affected by that science, those scientists, and this Society. No member, so far as I am aware, has proposed any action, or espoused any cause, that was not consonant in the highest sense with this objective. Our discussion is addressed to a fuller fulfillment of that objective.

Our discipline is today, more than at any time heretofore, a "many splendoured thing." It is a study of dimensions and depth undreamed a decade ago. We cannot now remotely imagine its probable proportions a decade hence; in technology alone, who dares guess what ultramicroscopy, tracer methods, axenic and tissue culture techniques, gnotobiotics, and other unprecedented tools of our times augur for the comparatively immediate future of our discipline? Moreover, parasitology has become of age: its frontiers are being relentlessly probed, and persons of all manner of disciplines and training are increasingly contributing their special knowledge and techniques to the elucidation of problems and phenomena. The sheer challenge of a way of life that characterizes what may be the majority of kinds and numbers of living things has captured imaginations everywhere. Parasitology, too, was born of interdisciplinary hybridization, and this is increasingly a hallmark of a growing, dynamic science. Many persons of training that was not fundamentally parasitological have become schooled, productive, and dedicated parasitologists. A further and significant evidence of maturity of our discipline is found, I think, in the solid concern

* Address of the retiring President, University Park, Pennsylvania, September 2, 1959.

that is widely manifested about the ravaging influences of parasites in agricultural production, wildlife conservation, and human health and welfare.

None can comprehend a subject so vast, and I have lightly confessed on occasions that a parasitologist is the last person who should be asked to define parasitology. Therein lie the opportunity and challenge, and therefrom stem the issues before us.

The Dimensions of Our Society

Good and reasonable men respond to good and reasonable causes. Parasitologists should think deeply upon, and explore ever more searchingly, the full dimensions of our discipline. In this endeavor, we should, I submit, affirm the common cause of all who contribute to it. By declared objective, our Society "fosters association of persons interested in parasitology and related sciences to improve teaching, promote investigation, and advance knowledge of these sciences." If we accept this as a true affirmation of objective, we are on a true course, and our Society, no less than our discipline, will grow in dignity, dimensions, and stature. Indeed, to the extent that our Society fulfills the needs for expression, the aspirations, the simple wont to association of persons of common scientific bondage, problems that vex will fade into insignificance. Our annual meetings and journal must be rededicated to fulfillment of these high purposes; unquestionably, we desire the best in both, yet this may mean that both could be more sensitively and efficiently adjusted to serve the needs of parasitologists in all facets of our discipline. This is not a plea for a large Society; nevertheless, a rededication to true purposes would inevitably result in healthy growth. I submit only that this is a good and reasonable cause.

The fate of great causes, it has been said, rests in small things. Should we not ask how well our Society serves those who are primarily devoted to such fields as human parasitology, veterinary parasitology, wildlife parasitology, and nematology? What about experimental parasitology, systematics, medical and veterinary entomology, acarology, protozoology? What about agricultural parasitology in its broader aspects?

So long as we are the *American Society of Parasitologists,* we can ill afford to be inflexibly orthodox or indifferent to any of our brethren who are dedicated to parasitological science. Rather, we should rededicate ourselves to the development of parasitology, with vigorous and vigilant regard for all facets and for all who have common cause with us.

Are there compelling reasons why our Society should foster a unified and balanced discipline? I have given it as my personal view that any other course is unthinkable! A moral science recognizes the worth of worthy contributions to it. The object of our Society and the aforestated common objective of service to all affected persons and interests impose a wholesome appreciation of all that wholesomely pertains to parasitology in its broadest sense. Our Society assuredly evolves as parasitology evolves. The dignity of our Society, no less than of our discipline, is hardly consonant with scientific myopia or anything short of the challenge and opportunity before it.

To this end, there is no place for favored disciplines and professions, but ample space for "togetherness" and "cross-fertilization;" no place for schism, since what pertains, belongs. The conventional tripod of parasitology, protozoan parasites, helminths, and arthropods that cause and carry disease, has surely, if nostalgically, become a myth; indeed, there is doubtfully today any basis, unless an arbitrary one.

for regarding any class of parasites as fundamentally different from any other, or for any reason alien to our Society interest.

When I began the formal study of biological science, I found growing glory and few frustrations in the simple credo that "anything that has to do with life is not foreign to biology." This concept imparts all there is of worth and gives the biologist something to live for! If parasitism is a way of life, can we afford to disallow that anything having to do with that phenomenon or that way of life is germane to parasitology?

If we will, we can collectively find the one best course to take. Perhaps one step would be an extension of affiliation for which our by-laws provide. There might be advantages in liaison with parasitological societies of other nations, including the large, active helminthological societies of Russia and Poland. These countries are named without political deference; yet, because science perceives no barriers, scientists seem unwittingly to bear, in part, the unwelcome duty of undoing much global damage that has been done by "man's inhumanity to man." Whether helpful from this standpoint or not, research parasitologists could unquestionably benefit from closer association with those who are primarily teachers, and the latter could gain by modest exploitation of research scientists about them. This mutualism would assuredly promote wholesome interest in science at critical age levels; it could also, as a significant bonus value, help toward solution of some probelms in training and recruiting research personnel. We should state in our Career Booklet or other publication exactly what this Society stands for. Possibly, a strong committee should make a forthright study of the dimensions of our Society from standpoints of the opportunity and challenge that confront it. Whatever the steps, if we allow that a few small ones might wisely be taken, there can be little doubt that the welfare of our Society, far more than that of our discipline, rests on the calibre and substance of expressions by individual parasitologists through the media of our meetings and publications; herein may be an individual challenge to subordinate self-interest to the larger interests and goals of our Society and discipline.

The Stature of Our Discipline

The future of parasitology will likely be shaped more by individual excellence than any other factor; and it is a safe prediction that individual competence in parasitology will be the rock of our discipline and that it will be in increasing demand. As a society of individuals dedicated to a common discipline of biological science, we may therefore infer that the collective excellence and competence of parasitologists as individuals will likely affect the stature and dimensions of both our Society and our discipline.

This would, indeed, be a happy moment if it could be spent in elaboration of these thoughts or of what has been mentioned about the challenge and importance of parasitology, and especially so if it could be devoted to what I believe to be the destiny and true place of parasitology, not only in biology as a mother science, but also in human society as the beneficiary. Unhappily, the problems that vex preclude excursions of this kind, although there can be no doubt that the evolution of parasitology in the United States has been closely paralleled by the evolution of our Society, and that the stature which both enjoy today is the product of individual excellence and competence. Nor can there be doubt that parasitology, seemingly more than our Society, owes all that it is today to the effort, imagination, and excellence of schooled,

productive, and dedicated parasitologists. It should be said, perhaps, that these attributes are those of parasitologists, but not identifiable with academic or professional degrees. This is, I think, the mark of a worthy science and also that of a learned, scientific society. Parasitologists have ever and always been predominantly individuals; and nothing, in either cause or effect, as far as I am aware, has ever been collectively proposed or espoused for special benefit to either our discipline, our Society, our members, or our colleagues.

Against the background of individual excellence and competence that has produced a unified, recognized science and a singularly unified society of workers, we would, I think, do well to develop and espouse sound, incontrovertible policy as a guide to collective behavior. It seems likely that circumstances will one day require, if that day is not already upon us, that we act as a Society in defense of the nobler objective that I have cited. There are clear reasons why parasitology should stand completely on its own feet, but the dignity of certain procedures within the framework of our Society is the paramount factor for consideration. We should certainly be ready to work and tighten our belts for any good cause. Indeed, as a case in point, our Society has outgrown the feasibility of imposing upon devoted parasitologists the routine of work on subscriptions, billing, membership, and editorial marking and proofing. These services will cost, yet the cost will be little compared to immobilizing professional people. But the character of our Society and discipline—the unselfish devotion to cause—must not be compromised in any effort, even if it should seemingly involve the preservation of our discipline. Here is a true dilemma, because parasitologists collectively, and especially this Society, cannot ignore their guardianship of our discipline yet they may not, organizationally, be able to effectuate this guardianship without apparent sacrifice of principle. There must be one best course to take, and there is room for confidence that problems can be solved by a simple statement of them to reasonable men. Three ought be mentioned because they bear upon the fate of parasitology as a science.

First, can parasitology fall into incompetent hands? No one having concern for parasitology can ignore the expressions of honest concern by some of the youngest in our ranks. The question implies that competence to do a job that has to be done may not always be the primary qualification for positions in parasitology. While a serious blow to parasitological research in any sector would be felt by parasitologists of all kinds throughout this country and the world, I have given it as my honest opinion that parasitology is destined to achieve ever greater stature. Honest mistakes may be made by responsible men who fail to weigh small things while contemplating achievement of the ultimate, and perhaps also by default on our part, yet, amid all the frustration and fiddle-faddle, honest search is apparently being made for competence in leadership, teaching, and research performance. I am more certain of the corollary to this, namely, that few persons will voluntarily accept an assignment for which they know they are wholly unqualified. I am optimistic, too, that an open statement on the issue in point is not only proper and, hopefully, reassuring, but also, at once, our most dignified and effective behavior.

Happily, the issue is not encountered by "nematologists"—our colleagues in the plant kingdom—or by wildlife parasitologists, or, directly, by a large proportion of parasitologists. Nevertheless, the issue is of as real concern to all as is the opportunity to inquire and teach or otherwise serve parasitology in any capacity. While specialists of many disciplines contribute nobly to the goals of better human and ani-

mal health, and many physicians and veterinarians have been dominant figures in one or another special discipline, it is inconceivable that we could become so muddled as to think that science and medicine are synonymous, or that scientific inquiry of any kind at any level is the special domain of individuals or classes of individuals having arbitrarily prescribed professional training. Assuredly, research positions in fields of science bear no direct relationship to degrees in medicine. To presume such would only deny first-rate research men the right of service to human and animal health. Indeed, it would undermine the whole fabric of science on which medicine itself depends. Parasitologists have always served the special needs of medicine and, unquestionably, they always will and should.

Scientific and other cultural research, on which the nobler welfare of today's world so much depends, and on which, as now seems, the fate of mankind precariously hangs, requires tolerance, comprehension, and vision to an unprecedented degree, especially in scientific and professional relationships. This could be the finest hour for parasitology!

Secondly, should parasitologists school themselves in shock absorption? All have frustrations and discouragements, and there have been and always will be problems. But our reference here is perhaps to the kind of thing that Warren Weaver called "dither" in a recent number of Science. When true issues and needs are obscured by our national scientific behavior, when we sense the seemingly advantageous and ephemeral awe of physical science, when good words have lost their meaning through corruption, when "glorification" of science, with its concomitant worship of so-called basic science, seems inextricably enmeshed with political objectives of both our own and other countries, when there is the overriding scientific obligation to prepare our country to play a determining role in the tragedy of world politics, when there are implications that our scientific and cultural welfare requires that we ape foreign methods and concepts—in such a time of national scientific groping, I submit that we can do much for our discipline by holding firm to the greater objective that I have stated, and perhaps much for our country by contributing what little we can to stability and balance. If scientific men are to remain free to indulge their scientific curiosities, it is plainly incumbent on them to dig deeper and perform better, yet to do these with some regard for the pertinence of their science to a better world.

Thirdly, does science have an obligation and goal of service? One small facet of this large question is the core of these remarks. I am indeed electing to emphasize a few related problems that seem to me to present a worthy challenge. There is a galaxy of problems like them. They pertain to what parasites do, which is of some personal interest, at least, because it has been my lot to be interested in what parasites do to us and what we can do to them. I do not mention certain problems as more important than others. Nevertheless, there is the likely possibility that parasitology owes something of its present recognition and stature to a greatly increased knowledge and concern about what parasites do to man, animals and plants. My thesis, then, is that problems in this area, although not scientifically defensible as more significant than problems in other areas, should not be neglected in the total program. Indeed, we should think in earnest about problems in this area since, in no mean measure, it is from these that flows the lifeblood of the entire discipline. This seems, for example, to fit the national pattern of federal grants for parasitological study, which in the year 1958 amounted to more than $12 million. Here is no argument that

the pursuit of knowledge should be motivated by usefulness of that knowledge in a utilitarian sense, yet it must be altogether apparent that we cannot condone the fashionable disdain for research that is useful. Indeed, unless we come to terms with ourselves on this point, I humbly confess that I can see no eventuality except a return to the dark ages. When science ceases to serve, our people must soon tire of it and, should this come to pass, we shall have entered scientific bankruptcy! Nor is this only a matter of funds; science is a commodity, but our world today would likely be much more interested in it than it is, were it not for the anti-social behavior and "other worldliness" of some of our articulate men of science.

What Parasites Do: Some Obscure Patterns of Fundamental Phenomena That May Be Significant to Parasitology and to Mankind

Six years ago, a few parasitologists undertook to evaluate losses caused by parasites of livestock in connection with a USDA appraisal of losses in Agriculture. The estimated billion dollar parasite-loss in farm animals included little of what might be called control costs, or intangible and indirect losses. It was mainly what might be called direct loss. Yet all of us were indelibly impressed with the complex ways in which losses are suffered from parasitism, and the paucity of information on the fundamental question of what parasites do. Indeed, with so much to be learned about problems in parasite biology whose solutions have potentially enormous significance to the field of parasite control, one cannot but sense some astonishment that so little attention has been given to these basic studies. If I had thought of reviewing at this time the chemical control of parasites, one sure alibi is that some of my good friends who advised me to do this promptly proceeded to do the job themselves. No one, however, except myself, has mentioned the most significant problem in the entire field of parasite control, namely, the development of efficient measures that circumvent or minimize dependence on foreign chemicals.

The aforementioned estimates were, I suppose, a product of theoretical research of a kind. My primary purpose on this occasion is to remind parasitologists, by reference to a few examples, of some patterns of potential significance of parasites which seem to be very sensible lines of investigation. They are admittedly ultilitarian and quite supplementary to the areas of imperfect knowledge that were enumerated in the aforenamed report. That report, however, seemed to have made it clear that new knowledge has a shattering impact on our sense of security regarding parasites; and accelerated researches have not only better oriented us to the issues of livestock parasitology, but have seemingly revolutionized accepted livestock standards of production, feed conversion, and performance. I should insist, too, that the lines of study that I mention are basic on grounds that they pertain to explorations into areas quite uncharted. They are deliberately confined to parasitic worms, and to these from standpoints only of what they potentially do.

The larval phase of nematodiasis. In 1933 (my best recollection), I was privileged to meet an extraordinary parasitologist who had just published a paper on acute hookworm disease in which significant mention was made of the "larval phase of uncinariasis." Bailey K. Ashford and I discussed the "obscure corner" of larval helminthiasis. We opined that the larval phase of helminthic infection would soon be recognized as a major cause of morbidity. We allowed for the established significance of larval tapeworm infections, about which much has been learned, and for the extensive recognition already given to a few conditions (e.g. trichinosis) that are largely attributable to larval invasion by helminthic parasites. We knew about creeping eruption and swimmer's itch. We did not know about visceral larva migrans, cerebrospinal nematodiasis, eosinophilic granuloma, and a few other conditions of "wandering nematode" etiology. These are extraordinary chapters, yet they seem only exemplary of many chapters that are assuredly unwritten about the larval phase of nematodiasis.

The specificity of parasites to their hosts, although a basic and useful concept, has somewhat blinded us to the apparently equally basic fact that immature parasites are generally less host-specific than mature ones. The limitations of parasitic exposure imposed by human habits, as well as livestock practices and animal behavior, are not likely of a kind to afford unusual discrimination concerning the kinds of larval parasites to which man and animals are exposed. My speculation, simply stated, is that man and animals are victims of invasion by infinitely more abundant and varied parasitisms than we have heretofore allowed, and that this multitude and magnitude of larval invasions, whether of normal or abnormal hosts, are almost certainly a significant, if yet largely unexplored, source of unrecognized and damaging parasitic diseases. There is, in any case, no certain defense for orthodox conceptions of parasitic diseases as functions of parasitic species that are regarded as specific or "normal" to one or more host species. Nor

should we think that an elaboration of this vast problem is unassociated with fundamental inquiry into current concepts of parasite specificity or of the operation of well-explored phenomena of parasitism. Several nematode species, for example, are already proved pathogens, even when comparatively or definitely mature, in hosts to which they are hardly accredited; and certain immunological phenomena, such as, for example, allergy and sensitization, have been found to be major causal factors in parasitic diseases. Unhappily, much must be unsaid about a vast subject of great potential importance to an understanding of parasitic disease, yet there is compensation in the conviction that most parasitologists will have already anticipated what is unexpressed and more than a few will have found support in their own researches for the patterns implied.

Helminths as reservoirs and vectors of disease. Although many ectoparasites, such as mosquitoes, tsetse flies, and fever ticks, are of primary and classical importance as biological vectors of serious diseases of man and animals, endoparasites have rarely been incriminated as vectors or reservoirs, notwithstanding the fact that they seem to fulfill determinable prerequisites for service in these capacities. There seems to be a lesson taught by a few exemplary studies on swine influenza and cholera, blackhead of turkeys, Newcastle disease of chicks, salmon poisoning of dogs, not to mention such human afflictions as lymphocytic choriomeningitis and poliomyelitis. Assuredly, there is still much to be learned about the spread of these diseases, and we should probably allow that there is incontrovertible evidence of helminthic intervention only in blackhead and salmon poisoning, yet the meager facts at hand seem surely to indicate that helminths and other endoparasites, no less than ectoparasites, are at least of potential significance in the propogation of many diseases of man and animals.

It is fitting to note that, except for a recent Presidential Address of outstanding merit on the subject of salmon poisoning, almost the only significant and recent work in this field has been done by Richard Shope. He published his first notable work on swine influenza two decades ago and is even now pursuing ascarids and hog cholera. One wonders why his must be a proverbial and prophetic voice crying in the wilderness.

Happily, there is support in this area from friends in nematology, whom I prefer to cite as colleagues in parasitology; they work with plant-parasitic nematodes and "soil sickness." The latter term, so far as I know, was coined by Gotthold Steiner, whom to know is to hold in high regard. In any event, it was Dr. Steiner's recognition of the great economic importance of nematodes to plant life that put nematology "on the map." Ironically, he was, and is, so pure a scientist as to be the last in the field whom one would have expected to be interested in practical problems. The fact is that he was interested in fundamental problems, even if they were practical, and in practical problems if they were fundamental.

It is seemingly appropriate to digress somewhat further in recognition of Dr. Steiner's predecessor in American nematology, that personable and gifted man of science, Nathan Augustus Cobb. Dr. Cobb lives eternally in the recollections of all who knew him. He directed attention to the ubiquity, abundance, variety, and habits of "nemas" that infest soil and plants, and gave zoological substance to nematology in this country. We should do well not to underrate today's significance of the fact that Dr. Cobb was a charter member and fifth president of this Society, and that Dr. Steiner was a charter member and sixteenth vice-president, and is still an active dues-paying member. Finally, the nematological vector studies from which I digressed are a part of the program of the Nematology Section of the Crops Research Division, U.S.D.A., and one individual identified with these investigations is a young parasitologist, now nematologist, A. F. Schindler, who began his career in the erstwhile Zoological Division.

Apparently, in the field of plant diseases, as in animal diseases, the concept of transmission by nematodes is not especially new, going back at least to 1930. Schindler has stated, however, that only recently, through efforts of several California workers, has transmission of a plant virus by nematodes been demonstrated. This work in 1958 incriminated the nematode *Xiphinema index* as a vector of grape fanleaf virus. There has also been significant work on relationship of nematodes to fungous and bacterial diseases of plant life.

This pattern of nematode relationships to plant diseases is exemplary not only of an area of great potential significance to our knowledge of what parasites do, but also of the indefensibility of schism within the discipline of parasitology.

Helminths as incitants of disease. This is a heterogeneous grouping of relationships of helminths to disease, yet the patterns are interrelated and quite apart from direct host-effects of helminthic invasion and activity, whether of larval or mature worms, and from phenomena of disease transmission. I have elected to refer to "helminths as incitants" merely to categorize phenomena wherein helminths are factors in the initiation and expression of disease.

A good example, although probably not the best, of the kind of parasite-disease relationship in point was published recently (July, 1959) in the Proceedings of the Helminthological Society of Washington. Therein an article by Loos dealt with nemic relationships to Fusarium wilt of bananas, a fungous disease which, for purposes of argument, I simply describe as probably of less concern to parasitologists of our Society than it is to banana growers. But I hasten to allow

that it is of major concern to some parasitologists. Perhaps I should also allow that my reference to this condition could, by some psychological twist, be associated with the fact that it is better known as "Panama Disease." At any rate, Loos showed that nemic infection of banana roots was not essential to the appearance of Panama Disease, and his findings revealed no evidence that nemas were vectors or reservoirs of the spores; he found, however, that a nematode with which he worked hastened the appearance of disease.

Another example of a relationship wherein helminths influence symptom-expression is found in black disease of sheep. This condition is not associated with nematodes, but rather with trematodes, the example being chosen for the reason that the disease does not occur except when liver flukes are present. It is interesting, if not altogether pertinent, that there seems to have been, since the classical work of Turner in 1930, a goodly number of parasitologists who readily proclaim that liver flukes are per se of little or no importance. Ordinarily, perhaps, we find that a scientific "breakthrough" is a stimulus to research endeavor, yet how many of us have sensed that notable achievements seem frequently to cause some abatement of researches in the particular areas. It may not have been until Olsen's work in the 1940's on cattle liver flukes that there was again serious scientific concern about liver flukes per se as pathogens in domestic ruminants and man.

A third example of the kind of parasite relationship under consideration, and one that should not be lightly considered, is the influence of parasitism on the initiation of tumors. Most of us will recall the excellent demonstrations by Bailey and his colleagues who have associated malignant tumors of dogs with the oesophageal worm, *Spirocerca lupi*. A review of the subject, namely, helminth parasites and neoplasia, by one who was also a colleague of Dr. Bailey, allowed that a proved relationship of this nature is found only in the instance of *Cysticercus fasciolaris* and hepatic sarcoma of rats. The first studies of this relationship were made by Borrel who published his most significant paper in 1906.

One further example of somewhat different nature may be permissible. With reference to swine influenza, Shope's hypothesis of lungworm transmission has been mentioned. Much more recently, however, a relationship has been emphasized between the expression of this disease and the exposure of young pigs to *Ascaris* larva. In this instance, controlled observations have shown that what is a comparatively mild infection per se progressed to severe virus pneumonia with high mortality in animals already victims of the larval phase of ascariasis. Those identified with the work emphasized the importance of parasite control as a measure for reducing losses from swine influenza.

Here is truly an "obscure corner" of helminthiasis. Undoubtedly, parasitism hastens the appearance of many nonparasitic diseases, or accentuates the expression of them, yet there is no clue to the specific role of parasites in any instance. It may be a simple or insignificant one, yet there may be complex relationships between or among specific agents, e.g. parasites and viruses, that influence the expression of malignancy or other condition of nonspecific etiology. In short, no one can today defensibly dissociate specific agents from culpability in the etiology and expression of so-called nonspecific diseases. Epilepsy from cerebral cysticercosis, not to mention cancers of virus etiology, are cause enough for quickening our concern about parasites in these obscure areas.

Helminthiasis as a stress factor. Stresses imposed by parasitism are not wholly separable from other parasitic influences on disease, but they are probably a definable category and potentially of great impact. Nematodirosis, experimentally benign in pure infections, is damaging in sheep that are suffering from haemonchosis; this may be an ill-defined example of the stress influence. A better example, although less well established, is the reported influence of ascarids on infectious bronchitis of chickens. Another may be the apparent relationship, noted by Chinese and Soviet workers, of schistosomiasis and other helminthiases to typhoid and paratyphoid fevers. There is also the allegation that symptoms of epidemic hepatitis in children are intensified by ascariasis, notably the larval phase of the disease.

The argument could possibly be strengthened by reciting more examples. It is only a speculation, however, that parasitism, even when seemingly benign, is altogether of a nature to aggravate and predispose to disease.

Nutritional interference by helminths. Many kinds of enteric parasitisms are commonly associated with distinct, often pathognomonic, lesions and symptoms which unquestionably affect digestion and assimilation. There is little knowledge about the specific effects of these parasitisms and a frustrating dearth of information on the importance of such effects. Yet, considering the "obscure corners," perhaps searching for specific influences by parasites on host nutrition, there appear to be influences of a kind that would go largely unsuspected unless demonstrated. The broad fish tapeworm, for example, reportedly robs its host of a vitamin. The poultry ascarid may reduce vitamin A level in the liver. Subclinical ascariasis of swine and trichostrongylosis of sheep have been identified with disturbed protein assimilation. No one knows the extent of this phenomenon or, let us say, the extent to which parasitism is instrumental in the protein deficiency of children in the tropics. Reference has already been made to the revolutionary

impact of new knowledge about parasites on feed-conversion data for livestock.

Nutritionists have emphasized a seeming universality of both general and specific effects of deficiencies; parasitologists might find reason to review the potential of such effects of parasitism, even by species that are commonly regarded as of little significance.

Helminthic activity and portals of entry of disease agents. Probably Ackert and his associates were the first to approach this subject experimentally. The fact of skin penetration by a wide variety of parasitic fauna suggests that even the most benign of parasites having this propensity and capability must inevitably assault a host's first line of defense in a manner favorable to the entrance of pathogenic organisms. If infections or morbidity commonly occur in consequence of such assault, we are in ignorance of a basic factor in the ecology and natural history of diseases. This could, in fact, be our plight. If they do not occur, we may be confronted with a missed phenomenon of protection.

There are, of course, many instances of secondary infection after parasitic invasion, as in demodectic mange and tick infestations. Some organisms (e.g. ticks) can inject toxins no less than specific agents of disease, already noted. There has long been presumptive association of staphylococcus infections with exposure to hookworm larvae. An uncertain relationship has existed between *Strongyloides* and foot-rot (*Clostridium* infection) of sheep. One of the most recent and significant instances of the pattern under consideration is the relationship of *Nippostrongylus* to Shope's fibroma, the pattern being not one of transmission, incitancy, or stress.

I was once admonished by a colleague that everyone knows that parasites are bad. While I have reservations about even that, I am sure that no one knows much about what parasites do. If a few examples have been drawn from "obscure corners" of this subject, it is only to suggest that we have a long way to go in "practical" parasitology. There is no presumption that anyone should neglect sound researches in order to explore patterns as described herein. "What parasites do" is, for example, not more significant than why parasites are parasites, or why parasites are fastidious about their hosts. Most of us, however, could probably be a little more alert to influences of parasitism on man, animals, and plants, or perhaps a little less enthusiastic about the beckoning "ivory towers," since the growth of parasitology will depend to some extent on the contribution that parasitologists make to a fuller appreciation of the biology of parasites in relation to human welfare.

CONCLUSION

I have said earnestly a few things about our Society and discipline. Although nought but speculation, they will have served a happy purpose if they cause some reflection on small things that make a great Society and discipline. I have said that parasitism is a way of life; that study of it has evolved a complex, important, and recognized scientific discipline, and also a great and solid scientific society; that all members of this Society are dedicated to what is best for parasitology, parasitologists, and the American Society of Parasitologists, and best also for all affected interests and persons; that nothing which has to do with parasitology is foreign to either our Society or our discipline; that neither can afford to compromise goals of individual competence and service, and that both have enormous opportunity and challenge; and, finally, that the future of parasitology is bright and secure because it will likely be shaped more by parasitologists than by their stars.

(To anyone interested, the author would be pleased to provide guides to some of the literature on "patterns" mentioned herein.)

RESEARCH NOTE

SOME PARASITES OF CENTRAL CALIFORNIA AMPHIBIANS.

During the winters of 1955 to 1958, inclusive, parasitological examinations were made of 178 amphibians collected in Marin and Sonoma counties, California. The findings reported herein are in addition to the writer's earlier presentation (1954, J. Parasit., **4**: 231). All host species contained at least 1 parasite and, in aggregate, 9 species of protozoa, 5 species of Trematoda and 2 species each of Cestoda and Nematoda were found. New host records are marked with an asterisk (*).

The animals examined follow. URODELA: (Ambystomidae), 7 adult and 22 larval *Dicamptodon ensatus;* (Salamandridae), 26 *Taricha granulosa* and 41 *T. torosa;* (Plethodontidae), 3 *Ancides flavipunctatus,* 17 *A. lugubris,* 26 *Batrachoseps attenuatus,* and 20 *Ensatina eschscholtzi.* SALIENTIA: (Ranidae), 11 *Rana boyli,* 4 *Rana aurora;* (Hylidae), 1 *Hyla regilla.* Unless otherwise indicated, all hosts are adults.

The parasites found and their hosts are:

PROTOZOA

Cepedea sp. from the rectum of 3 *Rana boyli.**

Cytamoeba bacterifera Labbé, 1894, in the erythrocytes of 1 *Ancides flavipunctatus**, 13 *A. lugubris**, 25 *Batrachoseps attenuatus**, and 7 *Ensatina eschscholtzi**. This parasite was also recovered in one of the adult (terrestrial) *Dicamptodon ensatus** but never seen in the aquatic larvae.

Endamoeba ranarum Grassi, 1879, in the cloaca and intestine of a single *Rana aurora.*

Hexamita ovatus Swezy, 1915, from the cloaca of 9 *Ancides lugubris,* 3 *A. flavipunctatus**, 13 *Batrachoseps attenuatus,* 2 *Dicamptodon ensatus**, 18 *Ensatina eschscholtzi,* 11 *Taricha granulosa* and 31 *T. torosa.*

Karotomorpha swezi (Grassi, 1926) in the cloaca of 26 *Batrachoseps attenuatus,* 22 *Taricha granulosa**, and 37 *T. torosa.*

Nyctotherus cordiformis (Eherenberg, 1838) from the cloaca of 2 *Taricha torosa**, and 2 *Rana boyli**.

Opalina sp. from the cloaca of 2 *Rana boyli** and a single *Hyla regilla.*

Tritrichomonas augusta (Alexieff, 1911) from the cloaca of one adult *Dicamptodon ensatus**, 4 *Rana aurora,* 11 *R. boyli**, 11 *Taricha granulosa** and 29 *T. torosa.*

Vahlkampfia sp. from the intestine and cloaca of 7 adult *Dicamptodon ensatus**, 2 *Ensatina eschscholtzi**, and 2 *Rana boyli**.

TREMATODA

Glypthelmins californiensis Cort, 1919, from the intestine of 8 *Rana boyli**.

Haematolechus sp. from the lungs of 1 *Rana aurora* and 8 *R. boyli**.

Halipegus aspina Ingles, 1936, from the stomach of a single *Rana boyli.*

Megalodiscus americanus Chandler, 1923, from the cloaca of 4 adult *Dicamptodon ensatus**.

Megalodiscus temperatus (Stafford, 1905) from the cloaca of 2 *Rana boyli**.

CESTODA

Bothriocephalus rarus Thomas, 1937, from the intestine of a single *Taricha torosa**.

Cylindrotaenia dianae (Helfer, 1948) n. comb. from the intestine of 7 *Batrachoseps attenuatus* and 3 *Ensatina eschscholtzi.* Helfer (1948, Tr. Am. Micr. Soc. **67**: 359–364) named this parasite *Proteocephalus dianae,* but morphological observations indicate that the correct genus should be *Cylindrotaenia* Jewell, 1919.

NEMATODA

Oxyuris dubia Leidy, 1856, from the cloaca of 2 adult *Dicamptodon ensatus**, 1 *Ancides flavipunctatus** and 3 *A. lugubris**.

Rhabdias sp. from the lungs of one *Dicamptodon ensatus** and 2 *Rana boyli.*

This work was supported, in part, by a grant in aid from the Society of the Sigma Xi.—
DONALD L. LEHMANN, *Department of Zoology, College of the Pacific, Stockton, California.*

THE RESISTANCE OF THE ENCYSTED FORM OF *TOXOPLASMA GONDII*

LEON JACOBS, JACK S. REMINGTON AND MARJORIE L. MELTON

U. S. Department of Health, Education, and Welfare, Public Health Service,
National Institutes of Health, National Institute of Allergy and Infectious Diseases,
Laboratory of Parasitic Diseases, Bethesda, Maryland

Studies on the transmission of *Toxoplasma gondii* have, in general, concerned the proliferative form of the parasite. These have resulted mainly in failures of attempts to obtain transmission by arthropod vectors, and in demonstrations of the fragility of free toxoplasmas (see Jacobs, 1956, for review).

On the other hand, there are data indicating that the encysted form of the parasite is more hardy. Jacobs (1953, 1956) contrasted the results of feeding tissues from chronically infected mice or rats to other mice with results of similar feedings of tissues from acutely ill animals. Since the former was much more successful than the latter, he hypothesized that the cyst form of the parasite, found in chronic infections, was better able to withstand the digestive process. Van Thiel and van der Waaij (1956) presented additional experimental work pointing to the same conclusion. The latter authors, in reviewing literature on feeding proliferative forms, conclude that the occasional positive results obtained are explainable by passage of the parasite through the buccal or pharyngeal mucosa, thus avoiding digestion.

In the case of Eichenwald's (1948) success in producing infections in newborn mice through the milk of acutely infected mothers, the proliferative forms may have survived in the stomach because of the low content of digestive enzymes in the newborn. However, milk of acutely infected mothers has not always produced infection as Cowen and Wolf (1950) reported. With 3 other strains of the parasite, we also have not found infection transmitted via the milk (to be published).

Frenkel (1949) and Rodhain and Gerebetzoff (1951) have described the cyst wall of *Toxoplasma* as argyrophilic, tough, and elastic. There has been some conjecture as to its origin. Originally it was presumed by many authors to represent the residual membrane of the infected cell, after the nucleus was pushed out by the developing mass of parasites. If the wall were of host origin, the structure would properly be called a pseudocyst. If the parasite contributed to the production of its enclosing membrane, as Frenkel and Friedlander (1951) hypothesized, the structure should properly be called a cyst. Lainson (1958) has presented morphological evidence indicating that cysts do not originate from masses of proliferating intracellular forms, but develop separately from small structures which increase in size and in number of contained parasites. It may be possible to test his observations experimentally and to explain the mechanism of growth of cysts. In the light of his report, it seems reasonable to adopt the word "cyst" rather than "pseudocyst" for the definitely walled structures found in animals chronically infected with *Toxoplasma*. This does not rule out the presence of dormant intracellular masses of parasites that

Received for publication June 12, 1957.

are not truly encysted and may be operative in the production of relapses of chronic infections such as are seen in the ocular disease (Jacobs, Fair, and Bickerton, 1954; Frenkel and Jacobs, 1958).

The bulk of the literature on the results of feeding experiments has been cited by van Thiel and van der Waaij (1956). Since a number of papers have appeared which postulate that human beings may acquire toxoplasmosis from meat (Siim, 1956; Weinman and Chandler, 1956), and since toxoplasmas have indeed been isolated from the flesh of swine (Jacobs and Melton, 1957a) and of sheep (Jacobs et al, to be published), it is important to devote attention to the ability of the *Toxoplasma* cyst to survive various conditions. The present report gives the results of tests on the survival of cysts when stored, when subjected to heat or to different ranges of pH or osmotic pressure, to freezing, and to the action of peptic and tryptic enzymes. The results of similar tests on the proliferative form of *Toxoplasma* will also be presented for comparison.

MATERIALS AND METHODS

Four strains of *Toxoplasma gondii* were used: The well-known RH strain, 2 strains recently isolated from swine, and the Beverley strain. The latter, originally isolated by Dr. J. K. A. Beverley, University of Sheffield, England, from a rabbit, was sent to us by Dr. Don E. Eyles.

In the early stages of this study (Jacobs and Melton, 1957b), the brains of rats with chronic infections with the RH strain were tested for infectivity after various treatments. The tissues were ground up in a mortar with saline, a sample was taken and kept at 4° C or 25° C for a control test of infectivity after the same length of time as the treatment interval of the test, and the rest was tested at intervals after the beginning of treatment.

The grinding up of soft brain tissue results in liberation of cysts. It could therefore be presumed that the environmental conditions to which the brain suspensions were subjected were operating directly on the encysted parasites. However, since we could not rule out the possibility that some cysts remained surrounded by brain tissue, we used only cysts isolated from tissue by means of a micro-isolation apparatus of the type described by Rees (1942). The cysts were easily picked out of brain suspensions by this instrument, and suspended in saline or other fluids. Microscopic observations could also be made directly on the immediate or short-term effects of digestive or other fluids on the cysts, and they could then be tested for infectivity.

Peptic digestive fluid was prepared as follows: Pepsin (Difco, 1: 10,000) 1.3 g, NaCl 2.5 g, HCl 3.5 ml, H_2O q.s. to 500 ml. In some tests peptones (Difco proteose peptone No. 3) were added to the digestive fluid to see if they exerted a protective effect on the parasites. The trypsin solutions were prepared from Difco trypsin, 1: 250, dissolved in 0.85 percent saline.

Infectivity was determined by inoculating the suspensions intraperitoneally into mice. With the RH strain, it could be expected that death of the mice would result from inoculation of even very small numbers of viable parasites. Therefore, the criteria used here were death and survival time. With the other strains of *Toxoplasma*, it was necessary to test the animals serologically if they survived. The mice were bled from the orbital sinus and routine dye tests were performed on a 1: 16 dilution of the serum. It was also possible, with the Beverley strain of the parasite, to study smears of the brains of test mice for the presence of cysts; if cysts were not found, the mice were tested serologically. The other strains of *Toxoplasma* did not produce cysts in such numbers that they were easily detectable on smears.

Details of individual experiments will be more conveniently described along with the presentation of the results.

RESULTS

Storage

Differences between cysts and proliferating parasites as to their viability after prolonged storage at 4° C were tested by comparing the infectivity to mice of the brains of rats with acute or chronic RH *Toxoplasma* infection. The acutely infected rats were killed 10 days after inoculation; the chronically infected animals had been infected 66 days prior to use. In one set of experiments, the brains of 6 rats from each group were ground in a mortar and suspended in a volume of pH 7.2 buffered

saline 10× their wet weight. The suspensions were passed through bolting silk to eliminate any large bits of tissue which could result in lack of homogeneity. They were kept refrigerated at 4° C thereafter and samples taken at intervals and inoculated into mice. The results are shown in table I.

It is presumed that although some cysts may have been present in the brains of the rats inoculated 10 days prior to use, the bulk of the parasites were probably proliferative forms. Also, it is believed that the chronically infected rats had mainly cysts. On the basis of these presumptions, the data in table I indicate a greater

TABLE I. *Effect of storage at 4° C on infectivity of brain suspensions*

Type of infection	Days stored	No. mice dead/no. inoculated	Average survival days
Acute	0	10/10	7.1
Chronic	0	10/10	7.1
Acute	7	5/9	9.2
Chronic	7	7/10	8.7
Acute	10	7/10	9.1
Chronic	10	9/10	16.0
Acute	14	1/10	10
Chronic	14	7/10	12.1
Acute	17	3/10	15.6
Chronic	17	9/10	10.5
Acute	21	1/10	4.0
Chronic	21	6/9	9.3
Acute	24	0/10	–
Chronic	24	6/10	10.0

TABLE II. *The effect on proliferative* Toxoplasma *of digestion with pepsin-HCl solution*

Volume of dig. fluid relative to tissue	Time of digestion (hours)	No. mice dead/no. inoculated	Average survival days*
1:10 (saline control)	0	3/3	5
1:10	1, 2, 3, & 4	0/20	–
1:100 (saline control)	0	4/4	4
1:100	1, 2, & 3	0/6	–
1:100 (saline control)	0	4/4	4.5
1:100	1, 2, & 3	0/6	–

* Computed from the days of death of mice succumbing to toxoplasmosis.

capacity of the cysts to survive storage. It is not possible definitely to equate the degree of infection of the two brain suspensions, and this would add a further qualification to the conclusion. However, since mice inoculated with the fresh suspensions, before storage, had very similar survival times, it appears that the numbers of organisms they received were comparable.

Additional tests, by the same methods, of brain suspensions from mice with chronic infections with the Beverley strain showed survival of parasites for as long as 68 days at 4° C.

Digestion in Pepsin

Table II shows the results of 3 tests on the effect of digestion of proliferative *Toxoplasma* in pepsin-HCl solution. The infective tissues were obtained from mice inoculated 4 days earlier with the RH strain of the parasite. The tissues used were liver, spleen, and lung.

As evidenced by the rapid time of death of mice inoculated with the organ suspensions prior to digestion, these tissues were parasitized with enormous numbers of toxoplasmas (Eyles, 1956). Even a 1-hour digestion resulted in complete loss of infectivity. Direct microscopic examination of proliferative forms introduced into digestive fluid revealed that they are immediately damaged; they become less refractile and more granular, and within 15–30 minutes appear "ghost"-like, with their outlines barely visible.

In contrast, tests of the infectivity of cysts of *Toxoplasma*, either in brain tissue or isolated from tissue, have shown that these forms are somewhat more resistant.

The results of experiments using brain suspensions from rats chronically infected with the RH or Beverley strains are shown in table III.

TABLE III. *Results of tests of infectivity of brain suspensions from chronically infected animals after peptic digestion*

Test no.	Source of parasites	Strain of Toxoplasma	Time of digestion (hour)	No. mice dead/no. inoculated	Average survival days
1	Rat	RH	0	10/10	7.2
		1:10 susp.	1	10/10	7.6
		in dig.	2	10/10	8.0
		fluid	3	10/10	11.6
			4	0/10	–
2	Rat	RH	0	4/4	8.3
		1:10 susp.	2	5/5	9.2
		in dig.	0	3/3	6.3
		fluid	2	3/3	9
3	Rat	RH	0	10/10	8.9
		1:10 susp.	1	10/10	11.1
		in dig.	2	5/10	10.4
		fluid	3	0/10	–
			4	0/10	–
4	Rat	RH	0	4/9	9.8
		1:100 susp.	1	8/10	10.5
		in dig.	2	0/10	–
		fluid	3	0/10	–
			4	0/10	–
5	Rat	RH	0	9/10	8.4
		1:100 susp.	1	5/5	7.4
		in dig.	2	0/5	–
		fluid	3	0/5	–
			4	0/5	–
				No. mice pos./no. inoculated	
6	Mouse	Beverley	0	2/2	(No deaths; infection determined by dye test and/or presence of cysts
			1	2/2	
		1:100 susp	2, 3	0/4	
		in dig.	4	1/2	
		fluid			
			0	2/2	
			1	2/2	
			2	1/2	
			4	0/2	
			0	2/2	
			1, 2, 3, 4 hr.	4/8 (1 of 2 mice per group)	

These data show that the cyst has a much greater ability to survive digestion by pepsin than does the proliferative form. Brain suspensions were sometimes infective after as long as 3 hours in the digestive fluid, although there was some loss of viability of the parasites by this time, as indicated by the longer survival time of the

inoculated mice. In order to learn to what part of the cyst this resisance could be attributed, and also to be certain, as mentioned above, that the cyst forms were not merely protected by brain tissue, the micro-isolation technique was used in further studies.

In the first series of micro-isolation tests, individual cysts from mice infected with the porcine or Beverley strains were placed in a small drop of saline in a flat dish (fig. 1). They were observed while the peptic digestive solution, previously

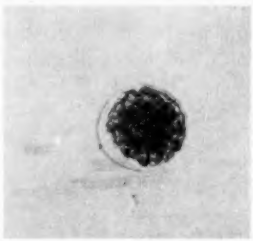


FIGURE 1. Isolated cyst of *Toxoplasma gondii*. Note cyst wall.

heated to 37° C, was added. The cyst wall was destroyed immediately on contact with the solution (fig. 2). The organisms liberated from the cysts were allowed to

FIGURE 2. *Toxoplasma* cyst immediately after exposure to pepsin.

settle for a few minutes, their location in the dish was marked, and the dish was transferred to a warm plate kept at 37° C, covered, and allowed to stand for various periods of time. At the end of the exposure period the dish was returned to the stage of the microscope, and the individual parasites were picked up in the micropipette, which had been cleaned with 95 percent ethyl alcohol and washed with saline in the interim. The contents of the micropipette were expelled into a tube containing 1 ml of saline, and this was then rapidly injected intraperitoneally into 2 mice, 0.5 ml each, by means of a conventional 1-ml syringe and 27-gauge needle. In addition to the digested organisms, parasites liberated mechanically from cysts by pressure from the micropipette, were similarly injected into mice as a check on their viability and on the effectiveness of the procedure.

Toxoplasmas liberated from individual micro-isolated cysts were thus demonstrated to remain infective for mice for up to 90 minutes in pepsin-HCl. No infections were obtained in mice injected with parasites from individual cysts after exposure to pepsin-HCl for 2 hours or longer.

In later work, micro-isolated cysts of the Beverley strain were introduced into tubes containing digestive fluids and were kept for various incubation periods. The tubes were then centrifuged, and the sediments were resuspended in saline and injected into mice. In all cases, cysts were also expelled into dishes containing the test solutions, so that they could be observed microscopically as well.

The viability of parasites released from cysts digested in pepsin-HCl, as determined by this procedure, is shown in table IV. In this experiment 5 separate tubes

TABLE IV. *Viability of suspensions of* Toxoplasma *from digested cysts as determined by inoculation of mice*

Time of digestion (hours)	% of peptone in digestive fluid	pH of digestive fluid	Pos. or neg. results in mice
1	0	1.5	+
	1	1.7	+
	2	1.9	+
2	0	1.5	+
	1	1.7	+
	2	1.9	+
3	0	1.5	–
	1	1.7	–
	2	1.9	–

TABLE V. *Effect of varied salt concentrations on the viability of isolated* Toxoplasma *cysts kept at room temperature (18°-20°C).*

% NaCl *	Time of exposure	Infectivity as determined by inoculating mice (+ or -)
0.1-2.1	2 hrs.	+
0.1-2.1	6 hrs.	+
0.1	12 hrs.	–
0.3-1.1	12 hrs.	+
1.3-1.7	12 hrs.	–
1.9	12 hrs.	+†
2.1	12 hrs.	–
0.1-2.1	24 hrs.	–

* The salt concentrations were 0.1, 0.3, 0.5% etc., in arithmetic progression to 2.1%.
† Only 1 of 2 tubes +.

of each of the test solutions were inoculated with 10 cysts each, and 2 mice were inoculated with the washed sediment from each tube after the incubation period. The parasites liberated from cysts were all infective to mice after 2 hours in the usual peptic digestive fluid, but not after 3 hours. The addition of 1 percent or 2 percent peptone to the fluid was tried to see if this added substance would increase the survival time of the parasites to 3 hours, in a manner similar to the increased survival of proliferative toxoplasmas in saline containing serum or neopeptone broth (Jacobs, Jones, and Melton, 1952). The wall of cysts in pepsin-HCl solution containing up to 2 percent peptone was digested within 45 seconds. The peptone obviously had no protective effect on the liberated toxoplasmas.

The time of dissolution of cyst walls in various concentrations of peptone in the pepsin-HCl solution and the effect of pH alone were determined. Direct microscopic examination revealed that at concentrations of 3 percent and 4 percent, pH 2.4 and 2.9, the cyst walls disintegrated in 3 to 5 minutes and 10 minutes, respectively. Cysts remained intact in pepsin-HCl solutions containing 5 percent to 10 percent peptone at pHs of 3 to 4.4; these cysts retained their infectivity to mice. The cyst walls disintegrated almost immediately in pepsin-HCl solutions adjusted with 0.1N NaOH up to pH 3.5; beyond pH 4.2 the cysts remained intact, and retained their viability at all levels for at least 2 hours, the longest period tested.

Digestion in Trypsin

Microscopic observation revealed that the wall of cysts exposed to 1 percent trypsin was immediately digested. Tests similar in design to those on pepsin were performed on parasites liberated by trypsin from cysts of the Beverley strain. A number of isolated cysts were placed into tubes containing a 1 percent solution of Difco trypsin 1 : 250 in saline and kept at 37° C. At various time intervals 2 tubes were centrifuged, the sediments resuspended in saline and recentrifuged, and then resuspended in saline and inoculated into mice. Infectivity of the parasites was demonstrated for up to 6 hours, the longest period tested. Proliferative parasites of the RH strain, similarly treated, survived in the trypsin solution for at least 3 hours. Tests were not made at 4 and 5 hours of exposure of proliferative forms to trypsin, but all were destroyed at 6 hours. These data were obtained from 4 experiments, comprising 47 tests.

Osmotic Pressure Changes

Cysts placed in distilled water remained intact, but were observed to undergo marked changes. Swelling of the wall occurred, apparently due to entrance of water, and the enclosed parasites became much lighter in appearance. In some experiments, exposure of cysts to distilled water for as short a time as 10 minutes rendered them non-infective to mice, but in other instances infectivity persisted for 30 minutes, but no longer.

In contrast, even low concentrations of saline (table V) preserved the viability of isolated cysts. The cysts were able to withstand all salt concentrations for up to 6 hours. Beyond 6 hours and up to 12 hours, the range of salt concentrations tolerated by the cysts was narrower; despite a single positive at 1.9 percent NaCl, it was probably 0.3 percent to 1.1 percent NaCl for this period. However, even at normal salt concentrations isolated cysts lost their infectivity on standing for 24 hours at room temperature. This is in contrast to the survival of cysts in macerated brain tissue at 4° C for 68 days.

Temperature

The effect of temperature on the infectivity of RH and Beverley strain cysts in suspensions of the brains of chronically infected rats is shown in table VI. This table records the results of a series of separate tests; so controls for each test are given separately.

In one test the infectivity of the cyst suspension was markedly reduced by a 1-hour exposure to 45° C, while in another experiment the cysts were still viable. In still a third test, with the Beverly strain, cysts withstood this temperature for an hour and a half. At 50° C and 56° C the cysts are vulnerable within shorter periods of time. Heating for 15 or 20 minutes, to attain a temperature of 60° C, was regularly lethal to the cysts.

In other tests, 2 brains of mice infected with the Beverley strain were divided in thirds. One anterior and one posterior third were examined microscopically for the presence of cysts, and the infectivity was proved by injection of mice. The other portions were frozen in a deep freeze at a temperature of about – 15° C. A portion from each brain was thawed at 24 and at 48 hours, and injected into mice. In all cases, freezing for these periods resulted in loss of viability of the cysts.

Brains of Beverley mice, infected with numerous *Toxoplasma* cysts, were kept in dishes and allowed to dry at 4° C, room temperature, and 37° C. After various periods of time, from 36 hours to 2 weeks, the dried tissue was macerated, suspended

TABLE VI. *Effects of temperature on viability of* Toxoplasma *cysts, as determined by infectivity to mice*

Temp. (°C)	Time	RH cysts No. mice dead/no. inoculated	Average days survival
5	1 hr.	5/5	
20	1 hr.	8/8	7.6
40	1 hr.	8/8	7.3
20	1 hr	6/8	9.0
45	1 hr.	1/8	8.0
20	1 hr.	8/8	8.3
45	1 hr.	7/8	8.3
50	10 min.	4/5	10
50	20 min.	4/5	8.5
50	30 min.	1/5	15.0
50	1 hr.	0/5	–
20	1 hr.	8/9	8.0
56	10 min.	0/6	–
56	20 min.	0/6	–
20 to 60	1 hr. 20 min.	5/6 0/8	6.6 –
to 60	20 min.	0/8	–
20	20 min.	15/16	8.0

Temperature (°C)	Time	Beverley cysts No. mice dead per mice inoc.	No. pos. serologically	% mice positive
50	15 min.	0/5	0	0
	30 min.	0/5	0	0
	1 hr.	0/4	0	0
to 60	15 min.	0/5	0	0
Control - many cysts				
45	1 hr.	1/2	0	50
	1½ hr.	4/4	–	100
(control) 25	1½ hr.	1/2	0	50
50	30 min.	0/4	2	50
50	1 hr.	0/4	0	0
50	1½ hr.	0/4	0	0
(control) 25	1½ hr.	2/2	–	100

in saline, and inoculated into mice. Even after 1 day at 18°–20° C, the brains were no longer infective. Only 1 positive result was obtained with a specimen dried for 72 hours at 4° C. Here there was evidence of considerable attrition of the cysts because only 1 of the 2 mice inoculated showed antibodies after 27 days and very few cysts were present in the brain of this mouse. On the other hand, control mice inoculated with the same amount of undried brain, died on the thirteenth day.

DISCUSSION

The failure of proliferative *Toxoplasma* to withstand the action of artificial gastric juice for even very brief intervals adds support to the conclusion of

Schmidtke (1956) and van Thiel and Van der Waaij (1956) that the occasional infection resulting from the feeding of proliferative parasites to animals probably results from the penetration of the buccal or pharyngeal mucosa by these forms rather than from their passage through the stomach. It is possible that there may occasionally be some survival of proliferative parasites in tissue when the gastric juice is low in acidity. Kozar et al (1952) took cognizance of this factor and found somewhat better survival of proliferative parasites in tissue when exposed to natural human gastric juices of low acidity as compared to highly acid juices; the exposure they used was one-half or one hour at 37° C. Schmidtke (1956) and Wildführ (1956) also found that proliferative *Toxoplasma* is destroyed most rapidly in gastric juice of low pH. Schmidtke also stated that these parasites survived longer in saliva and in intestinal juice. Although Wildführ infected hamsters by introducing parasites from the peritoneal exudate of mice directly into their stomachs by catheter, his results were best with the largest doses, and Kass (1952) could not produce toxoplasmosis in rabbits by a similar method. On the whole it appears unlikely that free parasites in the stomach could regularly succeed in invading the mucosa.

On the other hand, it is obvious that encysted parasites ingested with infected flesh could survive a normal period of digestion in the stomach. Furthermore, once they passed into the duodenum, they would have little difficulty in surviving even longer periods. The latter data agree with earlier unpublished observations made in our laboratory that, in mice infected with RH toxoplasmas by parenteral routes, parasites are easily demonstrable in the lumen of the upper parts of the small intestine.

The ability of cysts in tissues to withstand relatively long periods of storage and their greater resistance to changes in osmotic pressure also stand as evidence in favor of the possibility that the cyst is the more important form of the parasite as far as transmission is concerned. Lainson (1955) reached the same conclusion on the basis of the postulate that the cyst is the form observed when a balanced, chronic infection is reached between the parasite and the host.

In regard to heat resistance, however, the *Toxoplasma* cyst is not remarkable. A temperature of 50° C for 30 minutes was almost regularly lethal to the parasites in our tests, and 56° C for as short a time as 10 minutes rendered them non-infective to mice. The cyst is slightly more resistant than the proliferative form, which is killed by more than 5 minutes exposure to 50° C, according to Manwell and Drobeck (1951). In some of our tests an attempt was made to expose cysts to slow heating until a temperature of 60° C was obtained. This is the temperature at which a thermometer inserted into a roast of meat would inform the cook that the roast was now done "rare". Despite attempts at slow heating, this temperature was obtained in our small samples in 15 to 20 minutes, and in all tests the parasites were killed.

How these data relate to the epidemiology of human and animal toxoplasmosis is still not clear. Obviously, carnivores such as dogs, cats, wolves, foxes, etc. may acquire toxoplasmosis from the ingestion of infected tissue, and human beings who eat raw meat could be similarly infected. However, even strict vegetarians such as Hindus show serological evidence of experience with toxoplasmosis (Rawal, 1959; Jacobs, 1956) and the prevalence rates in various countries do not seem to be particularly related to the dietary habits in regard to meat (Jacobs, 1956). Lack of differences in prevalence of dye test reactions between the sexes has been reported

by Gibson et al (1956) and Feldman and Miller (1956). This would not add support to the hypothesis that meat is the main source of human infection. However, others such as Kozar (1958), Harboe (1952), Gronroos (1955), and Kudicke and Pohlig (1954), have reported that there are sex differences; i.e., women show higher prevalence rates than men. This may indicate that the habits and customs of various populations in regard to the handling and preparation of meat products may be important. Since some populations that do not consume much meat, and then only when it is well cooked, also have high prevalences of *Toxoplasma* antibodies, other factors may also be operative in the epidemiology of toxoplasmosis. The ability of the cyst to withstand the external environment may be a factor in the spread of *Toxoplasma* by some contaminative route, possibly with an invertebrate host as a mechanical carrier after it becomes infected from infected carrion. Such a mechanism could be important in the spread of infection to herbivorous animals.

SUMMARY

The cyst of *Toxoplasma* is capable of longer survival than the proliferative form when stored in infected tissues. Cysts of the Beverley strain survived as long as 68 days at 4° C. While proliferative forms, free or in tissues, are destroyed by peptic juice within a few minutes, tissue containing cysts remains infective for up to 3 hours. Parasites liberated by peptic juice from isolated cysts survive for 2 hours, even though the cyst wall is immediately destroyed. Proliferative forms survive exposure to 1 percent trypsin for at least 3 hours, but not as long as 6 hours. The cyst wall is immediately destroyed by trypsin; the liberated parasites survive at least 6 hours, the longest period tested. Cysts are rendered non-infective by exposure to distilled water for 30 minutes or less; even small concentrations of saline preserved their viability for longer periods. However, isolated cysts did not survive 24 hours in saline at room temperature. Cysts lose viability after 30 minutes at 50° C and 10 to 15 minutes at 56° C. There is some loss of viability after 1 hour at 45° C. Freezing and drying render the cysts non-infective.

REFERENCES

COWEN, D. AND WOLF, A. 1950 Experimental congenital toxoplasmosis. III. Toxoplasmosis in the offspring of mice infected by the vaginal route. Incidence and manifestations of the disease. J. Exp. Med. **92:** 417–429.

EICHENWALD, H. 1948 Experimental toxoplasmosis. I. Transmission of the infection in utero and through the milk of lactating female mice. Am. J. Dis. Child. **76:** 307–315.

EYLES, D. E. AND COLEMAN, N. 1956 Relationship of size of inoculum to time to death in mice infected with *Toxoplasma gondii*. J. Parasit. **42:** 272–276.

FELDMAN, H. A. AND MILLER, L. T. 1956 Serological study of toxoplasmosis prevalence. Am. J. Hyg. **64:** 320–335.

FRENKEL, J. K. 1949 Pathogenesis, diagnosis and treatment of human toxoplasmosis. J. Am. Med. As. **140:** 369–377.

——— AND FRIEDLANDER, S. 1951 Toxoplasmosis. Pub. Health Ser. Publ. No. 141.

——— AND JACOBS, L. 1958 Ocular toxoplasmosis. Pathogenesis, diagnosis, and treatment. A. M. A. Arch. Ophth. **59:** 260–279.

GIBSON, C. L., EYLES, D. E., COLEMAN, N. AND SMITH, C. S. 1956 Serological response of a rural Negro population to the Sabin-Feldman cytoplasm-modifying test for toxoplasmosis. Am. J. Trop. Med. Hyg. **5:** 772–783.

GRONROOS, P. 1955 Studies on *Toxoplasma* and the serology of toxoplasmosis. Ann. Med. Exp. Biol. Fenniae **33** (supp. 11). 113 pp.

HARBOE, A. 1952 Toxoplasma dye titers in 1600 blood donors in Oslo. Acta Path. et Microbiol. Scand., Supp. 93, pp. 325–330.

JACOBS, L., JONES, F. E. AND MELTON, M. L. 1952 The survival of *Toxoplasma gondii* in various suspending media. J. Parasit. **38:** 293–297.

——— 1953 The biology of *Toxoplasma*. Am. J. Trop. Med. Hyg. **2:** 365–389.

——— 1956 Propagation, morphology, and biology of *Toxoplasma*. Ann. N. Y. Acad. Sci. **64:** 154–179.

———, FAIR, J. R. AND BICKERTON, J. H. 1954 Adult ocular toxoplasmosis. Report of a parasitologically proved case. A. M. A. Arch. Ophth. **52:** 63–71.

——— AND MELTON, M. L. 1957a. A procedure for testing meat samples for *Toxoplasma*, with preliminary results of a survey of pork samples. J. Parasit. **43** (supp.) : 38–39.

——— AND ——— 1957b. Studies on the resistance of pseudocysts of *Toxoplasma gondii*. J. Parasit. **43** (supp.) : 38.

———, REMINGTON, J., MELTON, M. L. AND LUNDE, M. L. A survey of meat samples from swine, cattle, and sheep for the presence of *Toxoplasma*. J. Parasit. To be published.

KASS, E. 1954 Underskelser over *Toxoplasma* og Toxoplasmose. Akad. Trykningssentral, Olso. (Thesis).

KOZAR, Z. 1958 Investigations on the epidemiology of toxoplasmosis by means of the intradermal test. Acta Parasit. Polon. **6:** 225–249.

———, WYSOCKA, F. AND SIKORSKA, S. 1952 Studies concerning peroral infection with toxoplasmosis. Bull. State Inst. Marine and Trop. Med. Gdansk, Poland **4:** 37–39.

KUDICKE, H. AND POHLIG, W. 1954 Erfahrungen und statistische Beobachtungen uber den Serofarbtest nach Sabin und Feldman. Ztschr. Hyg. u. Infkr. **140:** 350–371.

LAINSON, R. 1955 Toxoplasmosis in England. II. Variation factors in the pathogenesis of *Toxoplasma* infections: The sudden increase in virulence of a strain after passage in multi-mammate rats and canaries. Ann. Trop. Med. Parasit. **49:** 397–415.

——— 1958 Observations on the development and nature of pseudocysts and cysts of *Toxoplasma gondii*. Trans. Roy. Soc. Trop. Med. Hyg. **52:** 396–407.

MANWELL, R. D. AND DROBECK, H. P. 1951 Mammalian toxoplasmosis in birds. Exp. Parasit. **1:** 83–93.

RAWAL, B. D. 1959 Toxoplasmosis. A dye-test survey on sera from vegetarians and meat-eaters in Bombay. Trans. Roy. Soc. Trop. Med. Hyg. **53:** 61–63.

REES, C. W. 1942 The construction of a micromanipulator for the isolation of protozoa. Am. J. Trop. Med. **22:** 487–492.

RODHAIN, J. AND GEREBETZOFF, M. A. 1951 Au sujet de la membrane limitant les pseudokystes des toxoplasmes. Compt. Rend Soc. Biol. **145:** 766–768.

SCHMIDTKE, L. 1956 Zur Uebertragung der Toxoplasmose durch Verfutterung von parasitenhaltigem Gewebe. Ztschr. f. Tropenmed. Parasit. **7:** 80–86.

SIIM, J. C. 1956 Toxoplasmosis acquisita lymphonodosa: Clinical and pathological aspects. Ann. N. Y. Acad. Sci. **64:** 185–206.

VAN THIEL, P. H. AND VAN DER WAAIJ, D. 1956 The significance of pseudocysts in the oral infection of man and animals with *Toxoplasma gondii*. Doc. Med. Geograph. Trop. **8:** 392–396.

WEINMAN, D. AND CHANDLER, A. H. 1956 Toxoplasmosis in man and swine. An investigation of the possible relationship. J. Am. Med. As. **161:** 229–232.

WILDFÜHR, G. 1956 Experimentelle Versuche zur Resistenz der Toxoplasmen. Ztschr. f. Hyg. **143:** 134–139.

RESEARCH NOTE

THE CHEMICAL COMPOSITION OF GREGARINE HARD-PARTS.

While engaged in studies on the cephaline gregarines of silverfish (Thysanura · Lepismatidae), Dr. Hilary Crusz observed that the newly formed spores (oocysts) of certain gregarines were colorless but darkened with age, suggesting a phenolic tanning process. He assigned me the task of investigating the chemical nature of these and other gregarine structures and kindly placed at my disposal his cultures of gregarine-infected silverfish. I am grateful to him and to Professor Wilfred Fernando, Head of the Department of Zoology, for the facilities given.

Gametocysts (102 to 115 microns) of *Garnharmia aciculata* from Australian specimens of *Ctenolepisma longicaudata* and spores (11.9 to 17 microns) of *Lepismatophila thermobiae* and *Colepismatophila watsonae* from local specimens of *Acrotelsa collaris* were investigated. Faecal debris was placed in a drop of water on a slide and gently crushed under a coverslip and examined through the microscope. Test reagents were introduced under the coverslip. When it was necessary to fix gametocysts for staining, crushed faecal pellets were fixed to the slide with albumen. At times even the much smaller spores were fixed in the same way.

According to Grassé (1953, Traité de Zoologie, Tome **1** (2)) the outer wall of the gametocyst is "mucilaginous" and according to Canning (1956, J. Prot. **3:** 54–55) it is gelatinous. Tests were first carried out on the gametocyst of *G. aciculata* in order to find its chemical nature. In water, the outer wall of the gametocyst was seen to be hard, translucent, and isotropic. Pressure on the coverslip did not put the gametocyst out of shape. On drying, this wall contracted into a thin, hardly perceptible skin round the granular central mass, which swelled back to its former diameter on the addition of a drop of water. Boiling water had no effect on it, but it dissolved in 0.2 percent HCl in about an hour's time and almost immediately in 0.2 percent NaOH solution. The biuret, ninhydrin and Sakaguchi's tests for proteins were negative and the xanthoproteic and Millon's tests were both negative and destructive. Sodium plumbite solution for sulphur was destructive without causing any blackening, nor was the gametocyst blackened by silver impregnation. In polarized light between crossed nicols it was found to be isotropic. In view of Grassé's statement, two important tests for muco-polysaccharides and mucoids were carried out, namely Hotchkiss' PAS reaction and Lison's toluidine blue technique. These were both negative. Dyes like methylene blue, gentian violet, picronigrosin, chlorazol black (in 70 percent alcohol) stained the gametocyst wall of which permanent preparations were made. The results of these various tests, although negative, therefore show that certain important substances like collagen, gelatin, muco-polysaccharides, mucoids, keratin, sclerotin, and chitin seem not to enter into the composition of the cyst wall.

Hardly anything is known about the chemical composition of the spores (oocysts). Spores of *Lepismatophila* and *Colepismatophila* were examined for this purpose. Tests given by Brown (1950, Quar. J. Micr. Sci. **91:** 331–339) for structural proteins, as well as further modifications of these tests, were carried out. Boiling water, dilute and concentrated acids (hydrochloric, nitric and sulphuric), dilute alkalies and 40 percent NaOH solution did not affect the spores in any way. Concentrated KOH made them lighter in color but did not dissolve them. All these tests were carried out for over 24 hours at room temperature up to boiling point. Sodium plumbite solution did not blacken the spores. Calcium chloride solution, 6M urea solution, and saturated calcium thiocyanate solution had no effect. One percent solution of sodium hypochlorite ("Milton," a stabilized commercial product, was used) dissolved spores within a minute. Ten percent solution was also used, as recommended by Brown. In this the spores dissolved even more rapidly. They were isotropic to polarized light.

According to Brown, "solution only in sodium hypochlorite solution suggests the presence of aromatic tanning," and also "when aromatic tanning is suspected sections should be cut of the tissues responsible for secreting the structure and these rather than the structures themselves should be subjected to histochemical tests." Tests for proteins and polyphenols on the darkened spores all proved negative.

The great insolubility of the spores (except in sodium hypochlorite solution) and their darkening with time suggest that they are composed of a sclerotin (quinone-tanned protein). Such a protein was first found in the ootheca of the cockroach (Pryor, 1940, Proc. Roy. Soc., London B **128:** 378–393) and later in the hard-parts of other animals. The evidence so far gathered as to the composition of gregarine hard-parts is wholly circumstantial. A detailed histochemical study of sections of gametocysts with their residual cytoplasm will have to be made before the substance of the spore wall of *Lepismatophila* and *Colepismatophila* is conclusively proved to be a sclerotin.—WINSTON E. RATNAYAKE, *Zoological Laboratories, University of Ceylon, Colombo, Ceylon.*

A SURVEY OF MEAT SAMPLES FROM SWINE, CATTLE, AND SHEEP FOR THE PRESENCE OF ENCYSTED *TOXOPLASMA*

LEON JACOBS, JACK S. REMINGTON AND MARJORIE L. MELTON

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

A number of workers have contributed data to or have expressed the hypothesis that meat may serve as a source of human *Toxoplasma* infection. Miller and Feldman (1953) and Feldman and Miller (1956) reported the occurrence of dye test antibodies in goats, cattle, swine, and sheep in various localities in the United States. Farrell et al (1952) and Momberg-Jorgensen (1956) reported toxoplasmosis in pigs in the United States and in Norway, respectively; and Varela et al (1956) in a study of ocular toxoplasmosis in Mexico, mentioned that they had found *Toxoplasma* in 87 of 817 pigs' eyes examined. Weinman and Chandler (1956) found *Toxoplasma* antibodies in a high percentage of swine fed on uncooked garbage in Connecticut, and a much lower prevalence among pigs fed on cooked garbage or grain. Sanger et al (1953) isolated *Toxoplasma* from cattle in Ohio. Olafson and Monlux (1942) described toxoplasmic encephalomyelitis in a sheep in New York; Wickham and Carne (1950) reported on a similar disease in a sheep in Australia; and Hartley (1957) has recorded perinatal mortality, due to *Toxoplasma,* in sheep in New Zealand. Roever-Bonnet (1957) performed a serological survey of 22 sheep, 29 cows, and 26 pigs at an Amsterdam slaughter-house and found 14 sheep, 6 cows, and 13 pigs with dye test titers of 1 : 20 or higher; only 11 animals, of the total of 77 studied, gave completely negative reactions. He also isolated *Toxoplasma* from the brains of 4 sheep, out of 30 animals whose brain and heart were inoculated into mice.

Siim (1956) mentioned the possibility that meat, milk, or eggs might serve as the sources of human toxoplasmosis. Van Thiel and Van der Waaij (1956) suggested that pseudocysts in meat or other animal tissues may be responsible for oral infection of man and animals. Weinman and Chandler (1956) presented an impressive case for incriminating undercooked pork as one of the important sources of human infection. Kayhoe et al (1957) mentioned that one of two cases of lympadenopathic toxoplasmosis they reported occurred in a woman who liked to eat raw meat.

It was pointed out by Jacobs (1957) that the main gap in the evidence thus far was the lack of information on the occurrence of cysts of *Toxoplasma* in the edible flesh of meat animals. On the basis of studies on the resistance of cysts of *Toxoplasma* to digestive juices (Jacobs and Melton, 1957a, Jacobs et al, to be published) and on the distribution of the parasites in the muscle of rats with chronic infections (Jacobs and Melton, 1957b), a survey procedure was developed for demonstrating *Toxoplasma* in meat samples (Jacobs and Melton, 1957c) and preliminary results were presented on pork and beef. It is the purpose of this paper to describe the procedures of our survey work in more detail, and to present data on sheep in addition to more complete data on swine and beef. Some corollary information on the

Received for publication June 12, 1957.

virulence of the *Toxoplasma* strains isolated, and some incidental findings on *Sarcocystis* will also be given.

MATERIALS AND METHODS

On the basis of our study of infections in the muscle of rats, it appeared that the diaphragm could be expected to be infected as frequently as other striated muscles. This made it convenient to choose the diaphragm as the meat sample to be investigated. The advantages were economic and administrative; there was no charge for the samples, and there were no memoranda required to justify the purchase of edible meats. The diaphragms of swine and cattle were provided free of charge by the Schluderberg-Klein Co. of Baltimore. We are grateful to Dr. Somers, Regional Office, U. S. D. A. in Baltimore, for making the arrangements with the S.-K. Company. We are also very thankful to the S.-K. Company for their kindness in transporting the specimens to Washington. The sheep specimens were obtained, also gratis, from the George R. Reid Co. of Baltimore.

The specimens were received fresh on the day of slaughtering or on the next day. They were either processed immediately or were packed in plastic bags and kept in ice for up to 3 days. Fat and fascia were stripped from the muscle, which was then ground in an ordinary household-type meat grinder. The trimmed hog diaphragm specimens ranged in weight from 48 to 137 g, with an average of 91 g. The beef specimens weighed from 55 to 205 g, with an average of 130 g; and the sheep specimens weighed from 12 to 113 g, with an average of 40 g. The ground meat was placed in a 2-liter flask and covered with a volume of peptic digestive solution about 10× its weight. The flask was placed on a magnetic stirring device in the hot room at 37° C, and digestion was allowed to proceed for 2 hours. After digestion, the suspension was filtered through several layers of coarse cheese cloth and centrifuged in 250-ml bottles for 15 minutes at 2000 rpm in the International No. 2 Centrifuge. The digestive fluid was poured off, the sediments were pooled into a bottle, resuspended in saline as a wash, and recentrifuged under the same conditions. Finally, the sediment was suspended in 10 to 20 ml of saline, depending on its volume, and inoculated intraperitoneally into 10 mice. Each mouse received 1 ml of the inoculum. Any residual suspension was kept in the refrigerator overnight and the same 10 mice received additional injections on the following day.

The mice were observed for 3 to 4 weeks, during which time any that died were examined by means of fresh smears of peritoneal exudate, if present, and Giemsa-stained impression smears of brain, liver, spleen, and lung. At the end of 1 month, about one-half of the survivors were bled to death or bled from the orbital sinus, and the serum specimens were tested at dilutions of 1:16 and 1:64 in the dye test for toxoplasmosis. In some but not all cases, if positive titers were obtained among these serum specimens, the remaining mice were killed and their tissues were subinoculated into fresh mice.

Estimations of the virulence of various strains of *Toxoplasma* isolated by these procedures were made by counting parasites from peritoneal exudates in a Neubauer-Levy counting chamber, and preparing appropriate dilutions in 10 percent serum-saline solution to give the desired numbers of parasites per ml. Standard amounts of these suspensions were inoculated intraperitoneally into mice, and the number of deaths and the survival times were noted.

RESULTS

Table I presents the results of examinations of 50 swine, 60 cattle, and 86 sheep. In some instances, serological tests were positive on the serum specimens of some of the mice, but *Toxoplasma* was not demonstrated in the remaining animals which were killed and subinoculated into fresh animals. In those cases in which *Toxoplasma* was demonstrated in the originally inoculated mice, not all of the mice were found positive. The same was true with second-passage mice. Apparently, the infections in the muscle were relatively light in most animals; and even though an attempt was made to prepare homogeneous suspensions of the digest, some of the inocula given to the mice did not contain parasites. Since our aim, however, was mainly to demonstrate *Toxoplasma* in these samples, once such a demonstration was accomplished no attempt was made to check closely all the mice inoculated with the same specimen. The low dye test titers found in some pools of blood from the mice may have been due to the dilution of a positive sample with a negative one.

TABLE I. *Results of studies on mice inoculated with digests of muscle from swine, cattle, and sheep.*

Animal	No. of specimens	Groups* of mice found positive		Total
		by dye test	by demonstration of parasites	
Swine	50	4**	8	12
Cattle	60	1†	0	1
Sheep	86	8	4††	8

* Ten mice (= 1 group) were inoculated from the digest of each specimen.
† One pool of serum from 2 mice gave a titer of 1 : 16. Two other pools, from 2 mice each, were negative serologically. No isolation was attempted from the remaining 4 mice.
** Isolation was not attempted.
†† Of the 8 groups of mice positive serologically, 6 were subinoculated into fresh mice, and in 4 of these groups toxoplasmas were demonstrated. The titers of the 2 groups negative on isolation were 1 : 16 (pools of serum from 2 mice each) ; the titers of the 4 groups positive on isolation were 1 : 1024 or higher.

Since numerous uninfected mice have been uniformly negative serologically, we believe these titers, although low, are indicative of infection with low numbers of organisms of an avirulent strain of *Toxoplasma*.

On the basis of the total number of samples found positive, either parasitologically or serologically, or by both methods, 24 percent of the swine and 9.3 percent of the sheep harbored encysted *Toxoplasma*. One positive dye test resulted from a pool of serum from 2 mice inoculated from 1 cattle specimen; thus the prevalence rate in these cattle specimens was 1.7 percent.

None of the strains of *Toxoplasma* isolated from sheep was virulent enough to kill mice of the original passage. On the other hand, 7 of the porcine strains killed all or some mice in the groups originally inoculated with them. Table II gives the results of tests of the virulence of some of the porcine strains, as compared to the well known RH strain. These data are more definite than percentages of kill of the originally inoculated mice, which could have varied with the intensity of infection in the samples. One of the strains tabulated, S-6, was highly virulent, producing death of mice as rapidly as the RH strain. The others were less pathogenic. Since in most cases the tests of virulence were made with parasites from chick embryos, which were infected as soon as possible after the parasites were demonstrated, these differences in virulence are considered natural, and not due to continuous passage through mice. The latter sometimes results in enhanced virulence of originally slow-growing strains, while little change occurs on passage in embryonated eggs (Jacobs and Melton, 1954).

TABLE II. *Comparison of porcine strains with the RH strain as to their virulence for mice.*

Strain	No. of mice dead/no. inoculated with designated number of parasites, and survival days		
	10	*100*	*1000*
S 2	—	10/12 (13.1)	11/12 (12.7)
S 4	17/20 (14.6)*	17/20 (13.5)	20/20 (11.9)
S 5	19/20 (12.3)	18/19 (10.9)	19/19 (10.8)
S 6	18/20 (8.7)	19/20 (7.8)	19/20 (7.2)
S 7	8/10 (13.1)	16/18 (11.7)	18/20 (10.3)
R H	10/10 (8.6)	10/10 (7.7)	10/10 (7.0)

* Figures in parentheses are the average survival days of the mice that succumbed to the infection.

One of the strains listed in Table II, S-2, was originally very slow-growing. It required 20 passages in cortisonized mice before an adequate number of parasites was available for use in a virulence test; it was not established in chick embryos until it had undergone 24 passages in mice. The data shown in table II are there-

fore not indicative of the original virulence of this strain which was very low. The strains isolated from sheep were similarly avirulent, and were not obtained in large enough numbers for virulence tests even after several passages in mice.

Microscopic examination of the washed sediment, after digestion and centrifuging, was carried out on the majority of the specimens. This was done with a 40X objective and 10X ocular. In no instance was *Toxoplasma* seen by this technique. However, a remarkable number of *Sarcocystis* infections were found. Of 39 hog specimens thus examined, 17 or 43.6 percent had *Sarcocystis*. All of the cattle and sheep digests were examined microscopically, and 98.3 percent and 97.7 percent, respectively, showed *Sarcocystis*.

In addition to the diaphragm of sheep, portions of the esophagus of some of these animals were examined macroscopically and with the use of the dissecting microscope in an attempt to find the Miescher's sacs. These were never seen, despite the finding of the individual spores in the digest. It is considered possible that the animals were too young, when slaughtered, for the *Sarcocystis* to have developed the large sacs described for *S. tenella* of sheep. This experience also suggests, however, that studies of the biology and transmission of *Sarcocystis* can be carried on more profitably by a digestion technique to reveal the parasites, than by attempts to see the Miescher's sacs in the muscle. The spores seen in the concentrated digest were motile and apparently unharmed by the treatment.

DISCUSSION

It is believed that the above results represent the first definitive demonstration of *Toxoplasma* cysts in the flesh of meat animals. They lend considerable support to the hypothesis that man can acquire the infection by consuming undercooked meat. While our sample of beef yielded only one positive serological result, it is believed that *Toxoplasma* may possibly be demonstrated to occur more frequently in other samples. It is to be remembered that Feldman and Miller (1956) found, in the same geographic locale, two herds of cattle with significant amounts of *Toxoplasma* antibody and two with none. It is quite possible that our small sample was derived principally from negative herds, and that a larger sample from more herds will reveal a higher prevalence rate for the parasite in this host.

Our studies of the effects of digestion on proliferative and encysted forms of *Toxoplasma* (Jacobs and Melton, 1957a, and Jacobs et al, to be published) indicate that the parasites demonstrated by the present technique were encysted organisms. The ability of these forms to survive a 2-hour digestion in artificial gastric juice furnishes additional support for the meat-to-man hypothesis of transmission. However, the importance of this avenue in the epidemiology of human toxoplasmosis requires evaluation in the light of certain serological data on human beings in various areas. For example, vegetarians in the United States have been shown to have *Toxoplasma* antibodies in relatively high titer (Jacobs, 1957). Also, there is as high a prevalence rate of serologic positives among Hindus, who are strict vegetarians, as among Moslems, who eat mutton (Rawal, 1959); indeed this rate is as high as that in the English midlands (Beverley et al, 1954). It seems obvious that, since herbivores acquire toxoplasmosis, there must be some route of transmission other than by the ingestion of infected flesh, and it is possible that *Toxoplasma* can also be acquired by humans by such a route.

It now becomes clear that more detailed studies are necessary to evaluate, in various geographic areas, the role of meat animals in the epidemiology of toxoplasmosis. The hypotheses of Weinman and Chandler (1956) of a rat-to-swine cycle, and a swine-to-swine cycle through the medium of uncooked garbage, require further study. Although these authors found the highest prevalences of *Toxoplasma* antibodies among swine fed uncooked garbage, epidemics of toxoplasmosis have occurred in localized herds of grain-fed swine as well. It may be possible to elucidate the life cycle of *Toxoplasma gondii* by studying local epidemics among herds of domestic animals.

The demonstrations of *Toxoplasma* in sheep and cattle were mainly on the basis of serologic tests of mice inoculated with the digests. The strains were so low in virulence that they did not kill the mice. On the other hand, some of the strains from swine were highly virulent and killed a high proportion of the mice inoculated with them. The variations found among strains of *Toxoplasma* isolated from swine were very striking. At the present time, all we know about differences in virulence, beyond the descriptions of pathogenicity for mice and other laboratory animals, is that these are correlated with invasiveness and rate of multiplication in tissue culture (Kaufman et al, 1958). It would be most interesting and important to understand what factors, if any, in the physiology of a host account for the differences in strains seen in nature. It would be most important, also, to investigate the possibility that the severity of the disease toxoplasmosis is correlated more with one source of infection than with another.

Beyond these points, our corollary data on the high prevalence of *Sarcocystis* in these meat animals are of considerable interest in regard to the serology of toxoplasmosis. No serologic survey of *Toxoplasma* antibodies in swine, cattle, or sheep has revealed any such high prevalence rate as we found for *Sarcocystis* by microscopic examination of the digests. Unfortunately, it was not possible for us to obtain blood specimens corresponding to the diaphragm specimens; so we cannot make a direct comparison here. However, there were obviously many *Sarcocystis* spores in the inoculum given to the mice, and these must have provided some antigenic stimulus. Despite this, the number of positive dye test reactions in the mice bears no relation at all to the number of samples found positive for *Sarcocystis*. Since *Sarcocystis* has been reported (Scott, 1943) to be very prevalent in meat animals, the cross-reactions between *Sarcocystis* and *Toxoplasma* related by Mühlpfordt (1951) and by Awad (1954) seem highly unlikely.

SUMMARY

By means of a peptic digestion technique for concentrating parasites from relatively large samples, a survey of *Toxoplasma* infection in diaphragm muscle from 50 swine, 60 beef cattle, and 86 sheep was conducted. After digestion and washing, the sediment from the digests was inoculated into mice. *Toxoplasma* was isolated from 8 samples from swine and 4 from sheep. In addition, 4 pork, 1 beef, and 4 mutton specimens produced dye test antibodies in the mice inoculated with them. Thus, 24 percent of swine, 1.7 percent of cattle, and 9.3 percent of sheep were demonstrated to have *Toxoplasma* infection. The parasite strains from swine varied from low to high virulence; those from sheep were all of low virulence. *Sarcocystis* was found in 43.6 percent of swine, 98.3 percent of cattle, and 97.7 percent of sheep.

The relation of these findings to the epidemiology of human toxoplasmosis and to the specificity of serological tests for this infection is discussed.

References

AWAD, F. I. 1954 The diagnosis of toxoplasmosis. Lack of specificity of Sabin-Feldman dye test. Lancet **267:** 1055–6.

BEVERLEY, J. K. A., BEATTIE, C. P. AND ROSEMAN, C. 1954 Human *Toxoplasma* infection. J. Hyg. **52:** 37–46.

FARRELL, R. L., DOCTON, F. L., CHAMBERLAIN, D. M. AND COLE, C. R. 1952 Toxoplasmosis I. *Toxoplasma* isolated from swine. Am. J. Vet. Res. **13:** 181–185.

FELDMAN, H. A. AND MILLER, L. T. 1956 Serological study of toxoplasmosis prevalence. Am. J. Hyg. **64:** 320–335.

HARTLEY, W. J. 1957 Toxoplasmosis as a cause of ovine perinatal mortality. N. Z. Vet. J. **(Dec.):** 119–124.

JACOBS, L. 1957 The interrelation of toxoplasmosis in swine, cattle, dogs, and man. Pub. Health Rept. **72:** 872–882.

——— AND MELTON, M. L. 1954 Modifications in virulence of a strain of *Toxoplasma gondii* by passage in various hosts. Am. J. Trop. Med. Hyg. **3:** 447–457.

——— AND ——— 1957a Studies on the resistance of pseudocysts of *Toxoplasma gondii*. J. Parasit. **43** (supp.) : 38.

——— AND ——— 1957b The distribution of *Toxoplasma gondii* in the muscles of rats with chronic infections. J. Parasit. **43** (supp.) : 41–42.

——— AND ——— 1957c A procedure for testing meat samples for *Toxoplasma*, with preliminary results of a survey of pork and beef samples. J. Parasit. **43** (supp.) : 38–39.

———, REMINGTON, J. S. AND MELTON, M. L. The resistance of the encysted form of *Toxoplasma gondii*. J. Parasitol. **46:** 11–21.

KAUFMAN, H. E., REMINGTON, J. S. AND JACOBS, L. 1958 Toxoplasmosis. The nature of virulence. Am. J. Ophth. **46:** 255–260.

KAYHOE, D. E., JACOBS, L., BEYE, H. K. AND MCCULLOUGH, N. B. 1957 Acquired toxoplasmosis. Observations on two parasitologically proved cases treated with pyrimethamine and triple sulfonamides. New Eng. J. Med. **257:** 1247–1254.

MILLER, L. T. AND FELDMAN, H. A. 1953 Incidence of antibodies for *Toxoplasma* among various animal species. J. Inf. Dis. **92:** 118–120.

MOMBERG-JORGENSEN, H. C. 1956 Toxoplasmosis in pigs. Nord. Vet.-Med. **8:** 227–238.

MÜHLPFORDT, H. 1951 Das Verhalten Sarcosporidien-infizierter Tiere im Serofarbtest auf Toxoplasmose nach Sabin-Feldman. Ztschr. Tropenmed. Parasit. **3:** 205–215.

OLAFSON, P. AND MONLUX, W. S. 1942 *Toxoplasma* infection in animals. Cornell Vet. **32:** 176–190.

RAWAL, B. D. 1959 Toxoplasmosis. A dye test survey on sera from vegetarians and meat eaters in Bombay. Trans. Roy. Soc. Trop. Med. Hyg. **53:** 61–63

ROEVER-BONNET, H. DE 1957 Toxoplasmosis in slaughter-cattle, particularly in sheep. Doc. Med. Geograph. Trop. **9:** 336–338.

SANGER, V. L., CHAMBERLAIN, D. M., CHAMBERLAIN, K. W., COLE, C. R. AND FARRELL, R. L. 1953 Toxoplasmosis V. Isolation of *Toxoplasma* from cattle. J. Am. Vet. Med. Assoc. **123:** 87–91.

SCOTT, J. W. 1943 Economic importance of Sarcosporidia, with especial reference to *Sarcocystis tenella*. Univ. Wyo. Agr. Exp. Sta. Bull. No. 262.

SIIM, J. C. 1956 Toxoplasmosis acquisita lymphonodosa: Clinical and pathological aspects. Ann. N. Y. Acad. Sci. **64:** 185–206.

VAN THIEL, P. H. AND VAN DER WAAIJ, D. 1956 The significance of pseudocysts in the oral infection of man and animals with *Toxoplasma gondii*. Doc. Med. Geograph. Trop. **8:** 392–396.

VARELA, G., ROCH, E. AND TORROELLA, J. 1956 Estudio de toxoplasmosis ocular. Rev. Inst. Salub. y Enferm. Trop. **16:** 17–19.

WEINMAN, D. AND CHANDLER, A. H. 1956 Toxoplasmosis in man and swine. An investigation of the possible relationship. J. Am. Med. As. **161:** 229–232.

WICKHAM, N. AND CARNE, H. R. 1950 Toxoplasmosis in domestic animals in Australia. Austral. Vet. J. **26:** 1–3.

GLOBIDIUM INFECTIONS IN THE SUDAN WITH SPECIAL REFERENCE TO *GLOBIDIUM GILRUTHI* (CHATTON, 1910) IN SHEEP AND GOATS

K. N. SOLIMAN, PH.D., D. V. M. & S.

Research Division, Department of Animal Production, Khartoum, Sudan

The first known *Globidium* on record was that observed by Flesch (1883) in the small intestine of the horse and named by him (1884) *Globidium leuckarti*. Following this finding, similar parasites were described by different authors affecting the kangaroo, sheep, goat, wallaby, wombat, armadillo, and camels in different parts of the world. Species recorded have been given various names. Wenyon (1926) classified them together in the single genus *Globidium*. Members of this genus so far described affecting farm animals include the following: *G. leuckarti* Flesch, 1883, in the small intestine of the horse; *G. gilruthi* (Chatton, 1910), in the abomasum and small intestine of sheep and goats; *G. besnoiti* (Marotel, 1912), affecting skin and ileum of cattle, and skin and epiglottis of horses; *G. cameli* Henry and Masson, 1932, in the ileum of the camel.

Globidium infections were first observed in Sudan by Bennett (1927), who reported a case which he provisionally diagnosed as a peculiar sarcosporidial infection in a horse. Further investigation of mounted sections from this case proved the parasite in question to be *Globidium*. In 1933 the same author described 3 more cases, 2 in horses and 1 in a bull along with 2 suspected cases in a horse and a donkey. Detailed clinical and histopathological study of material from these cases gave evidence of the close conformity of this condition to that described in cattle by Besnoit and Robin (1912) and which was named *Sarcocystis besnoiti* in the same year by Marotel.

The occurrence of another species of this genus, namely, *G. gilruthi* (Chatton, 1910) affecting sheep and goats in Sudan is recorded here.

MATERIALS AND METHODS

A total of 425 sheep and 240 goats from different parts of Sudan was examined from June, 1956, to June, 1958. The intestinal tract of each animal was opened longitudinally by a pair of bowl scissors, cleaned of its content, and examined carefully. A number of smears from each positive case were prepared from crushed cysts and a few were stained with each of the following stains: haematin, fuchsin, Romanowsky, and Giemsa. In order to study the relation and distribution of this infection in the invaded tissue elements, as well as to follow the parasite in its developmental cycle, pathological material was collected from 20 infected sheep and from 20 goats. This comprised pieces from the mucous folds, wall of the abomasum, and small intestine. Sections from the 40 cases were stained with hematoxylin-eosin.

OBSERVATIONS

The first *Globidium* was encountered in the digestive tract of a goat in June, 1956, during a systematic parasitological examination of slaughtered animals in the Khartoum abattoir. Macroscopic examination of the abomasum showed its inner surface to be studded with a number of round, white, opalescent cysts embedded in

Received for publication October 24, 1958.

the mucosal lining. Some of these cysts were empty and gave the impression of small round ulcers with white raised edges and distinctly hollow centers. Although the infection was not a heavy one, it extended throughout the small intestine in the form of odd, scattered cysts along its mucosal surface. These cysts were of pinhead size, having a diameter of approximately 0.5–1.0 mm, with convex surfaces slightly elevated above the level of the surrounding normal mucous membrane.

Mucous scrapings of the intestine as well as fecal examinations proved negative for coccidiosis. Examination of stained smears from crushed cysts revealed large numbers of spores of almost uniform morphology and size, each banana-shaped, pointed at one end and blunt at the other. Individual spores had a nucleus at the blunt end and a distinct granule towards the sharp hyaline end, and measured 10 by 1.8 microns. The spores did not stain with the same intensity, probably due to difference of age in individual cell elements. Mounted sections stained with hematoxylin-eosin proved these cysts to be fully developed *Globidium gilruthi* (Chatton, 1910).

Later it was found that 32 percent of the sheep and 40 percent of the goats examined were infected with *Globidium*. Triffit (1925) recorded *G. gilruthi* infection in 92 percent of sheep in England. Sarwar (1951) reported the occurrence of this protozoan parasite from the Indo-Pakistan sub-Continent, where 34 percent of sheep and goats slaughtered at Lahore, and 94 percent in other localities of East Punjab, were infected. Soliman (1958) reported *G. gilruthi* in sheep and goats in Egypt with a minimal infection rate of 18 percent in sheep and 28 percent among goats slaughtered at Cairo abattoir. In none of the animals examined in Sudan was the infection limited solely to the small intestines as described by Moussu and Marotel (1902) and by Marsh and Tunnicliff (1941). In 79 sheep and 45 goats the infection was confined to the abomasum, while in the remaining 57 sheep and 51 goats the small intestine was infected as well. The infection was more frequently seen in the duodenum and jejunum than in the ileum.

Hemorrhages such as mentioned by Wenyon (1926) and Hagan (1957) were not encountered. Richardson (1948) stated that no pathogenicity has been attributed to *G. gilruthi* infection. Heavy infections were found among emaciated sheep and goats, but other more probable debilitating factors, such as haemonchosis, were associated with the infections. Some heavy infections were also observed in rather healthy animals. It seems essential to investigate further the possible pathogenicity of this organism before its clinical importance can be assessed.

As to topographical distribution, the parasite was found in sheep and goats in almost all places visited in Khartoum, Kordofan, Blue Nile, Upper Nile and Equatoria provinces. Although the number of each species of animals examined in some of these areas was too small to allow a comparison of infection rate, it was sufficient to establish the existence of infection.

In 40 cases cysts were observed in between mucosal cells of the abomasum and small intestine at different depths from the free mucosal surface. Immature and mature cysts were observed resting on the longitudinal muscle fibers of the muscularis mucosae with their upper borders 215 microns below the free surface, while some mature cysts with diameters of 380 to 559 microns were just a few microns from the free surface. The cyst wall had 2 clear zones: an inner one staining homogeneously with concenteric striations 3 to 4 microns in thickness, and an outer more

lightly stained one 3 to 6.6 microns in width with perpendicular striae. No definite cell structure could be identified in the cyst wall. Completely mature cysts filled with enormous numbers of spores were encountered in the majority of cases. Spores in sections of these cysts were so packed that free ones were not easy to find, but when observed appeared rather morphologically identical to others in smears from crushed cysts. Each was a sickle-shaped cell with a nucleus at the blunt end. The other end of the spore was sharp and hyaline with a distinct granule between it and the nucleus of the cell. These spores ranged from 6 to 9 microns in length and from 1.2 to 1.8 microns in width. These measurements were less than those observed in smears from crushed cysts, probably because of shrinkage produced by the fixative. A few cysts revealed the presence of a large nucleus-like structure situated at one inner border of the membranous capsule measuring 15 to 25 microns by 70 to 80 microns. Some ruptured cysts were encountered in few cases where the spores were set free. The ruptured border of the cysts was always the one towards the free mucous surface of abomasum or small intestines. No development has been observed in any of these spores in such sections, and no cell invasion by such spores was recorded in any of the material examined.

Occasionally cysts in different stages of maturity were seen. The young forms, spherical cytoplasmic bodies with large numbers of deeply stained nuclei situated on the outer surface of each, constituted the sole elements filling some of these cysts. Such cytoplasmic spherules when cut through the middle looked like empty circles with nuclei all around their circumferences. Transitional stages of development between these cysts and the fully mature were filled with free spores and groups of still attached spores to cytoplasmic bodies. None of the sections examined revealed merozoites of coccidial infection.

Most of the *Globidium* cysts encountered in sheep and goats in this study were fully mature ones. In few of the cases, earlier stages of development, similar in their description to those quoted by Wenyon (1926) from Chatton's original (1910) record of *G. gilruthi,* were observed. Finding the different stages of development, as already reported in this work, leaves no doubt that the protozoon observed in sheep and goats in Sudan conforms to the classical description of *G. gilruthi* (Chatton, 1910).

SUMMARY

Globidium gilruthi infection in sheep and goats in Sudan is here recorded for the first time. Study of 665 cases over a period of 2 years showed that 32% of 425 sheep and 40% of 240 goats were infected. Cysts were observed in the abomasi of all the infected animals and in 57 sheep and 51 goats in the small intestines as well. The lesions caused by *G. gilruthi* are described. The infection was met with in all the areas visited at Khartoum, Kordofan, Blue Nile, Upper Nile and Equatoria provinces.

ACKNOWLEDGMENTS

This artice is published with the kind permission of Dr. Ibrahim Khalil, Director of the Department of Animal Production, Khartoum. Thanks are also due to Dr. Amin El Karib, Chief Veterinary Research Officer, Khartoum, for his interest in the subject.

LITERATURE CITED

BENNETT, S. C. J. 1927 A peculiar equine sarcosporidium in the Anglo-Egyptian Sudan. Vet. J. **83:** 297–304.

———1933 *Globidium* infections in the Sudan. J. Comp. Path. **46:** 1–15.

BESNOIT, C. AND ROBIN, V. 1912 Sarcosporidiose cutanée chez une vâche. Rev. Vet. **37:** 649–663.

CHATTON, E. 1910 Le kyste de Gilruth dans la muqueuse stomacale des ovides. Arch. Zool. exp. et gen. **45**, 5. s., **5**, Notes et Rev. (4), pp. cxiv–cxxiv.

FLESCH, M. 1883 Ueber ein Sporozoon beim Pferde. Zool. Anz. **6:** 396–397.

———1884 Ueber einen Parasiten in der Darmwand des Pferdes. Mitth. Naturf. Ges. Bern (1076–1080), 1. Heft, Abhandl. pp. 26–59.

HAGAN, W. A. 1957 The Infectious Diseases of Domestic Animals. Comstock Publishing Associates, New York.

MARSH, H. AND TUNNICLIFF, Z. A. 1941 Enteritis in sheep caused by infection with the protozoon parasite *Globidium gilruthi*. Am. J. Vet. Res. **2:** 174–177.

MOUSSU, Y. AND MAROTEL, G. 1902 La coccidiose du mouton et son parasite. Arch. Parasit. **6:** 82–98.

RICHARDSON, U. F. 1948 Veterinary Protozoology. 1st ed. Oliver and Boyd, Edinburgh.

SARWAR, M. M. 1951 Occurrence of *Globidium gilruthi*, a protozoon parasite of sheep and goats, from Indo-Pakistan sub-continent. Parasitology **41:** 282.

SOLIMAN, K. N. 1958 *Globidium gilruthi* (Chatton, 1910) infection in the digestive tract of sheep and goats in Egypt. Parasitology **48:** 291–292.

TRIFFIT, M. J. 1925 Observations on *Gastrocystis gilruthi*, a parasite of sheep in Britain. Protozoology **1:** 2–18.

WENYON, C. M. 1926 Protozoology. Vol. I. Bailliere, Tindall and Cox, London.

RESEARCH NOTE

ON THE CONTINUOUS CULTURE OF OPALINIDS.

An egg slant medium (Yang and Bamberger, 1953 Science **118:** 252) has been used to culture continuously *Opalina* spp., isolated from *Bufo valiceps*, for more than 6 years. A more convenient liquid medium has now been developed. The medium which has supported the growth of the opalinids for the last 2 years, contains the following: NaCl, 4 g; Na_2HPO_4, 1.1 g; KH_2PO_4, 0.3 g; $NaHCO_3$, 0.4 g; KCl, 0.2 g; $CaCl_2$, 0.05 g, $MgSO_4$, trace; liver concentrate (Wilson's), 5 g; and enough distilled water to make 1 liter. Four ml of this solution were added to each test tube and the tubes were plugged with cotton and autoclaved. Before inoculation, 0.1 ml of filtered, inactivated human serum was added to each tube. Since bacteria grew rather poorly in the liquid liver concentrate medium, the culture could be transferred weekly or biweekly. Good growth of the opalinids has been found in cultures allowed to stand in room temperature for a period of a month. The addition of antibiotics to the medium was not necessary.

Fresh isolates of opalinids may be inoculated on either egg slant medium or liquid liver concentrate medium to which antibiotics have been added (penicillin G sodium, 2000 units, or dihydrostreptomycin sulfate, 20 mg, or both). The opalinids can be freed from other protozoa by serial transfers using the overlay of the egg slant medium as the isolation medium. Contamination with small flagellates was, at times, most troublesome as they multiplied very rapidly in the culture medium and eventually crowded out the opalinids. Attempts to start the culture with a small number of opalinids in either medium have not been successful.—WILLIAM C. T. YANG, *Department of Pharmacology and Toxicology, School of Medicine, University of Southern California, Los Angeles 7, California.*

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: AMINO ACID PERMEATION INTO TAPEWORMS FROM ELASMOBRANCHS*

CLARK P. READ,** JOHN E. SIMMONS, JR.,** AND ALVIN H. ROTHMAN

Marine Biological Laboratory, Woods Hole, Massachusetts, and Department of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

The most fundamental aspects of parasitism are those concerned with nutrition. Parasitism is most frequently defined in nutritional terms. The penetration of materials of potential nutritional importance from the environment into the tissues of a parasite, therefore, constitutes a class of phenomena of first importance in parasitism.

The general problems involved in differentiating simple diffusion, facilitated diffusion or transport, and active transport through living membranes are complex, and have been reviewed in recent years by Davson and Danielli (1952), Danielli (1954), Rosenberg (1954), Patlack (1956), Christensen (1958), Scheer (1958), and others.

There are no critical studies of facilitated diffusion or active transport of organic substances into animal parasites. The studies of Daugherty (1957a, b) and of Daugherty and Foster (1958), purporting to demonstrate active transport of certain amino acids into *Hymenolepis* and *Raillietina,* are completely inadequate in this respect. No kinetic data were presented by these authors, and the stereospecificity observed can be explained by the assumption of weak sites of binding within the animal. Further, the effects of temperature on the amount of substance found in the worms after a fixed period of time are quite unreliable in determining how the material entered. With nonliving membranes, Q_{10} values for diffusion may range from slightly above 1.0 to more than 40 (Davson and Danielli, 1952).

On the other hand, Trim and Alexander (1949) carried out an excellent study of the kinetics of penetration of various anthelminthics through the cuticle of *Ascaris.* These drugs apparently enter by diffusion through a lipoid membrane.

Tapeworms seem to represent unusually good material for studies of permeation. Since they lack a digestive tract, substances must enter the animal through the external surface. Tapeworms are of sufficient size so that they may be removed from test solutions after relatively short periods of time and utilized in studies of kinetics. The experiments to be reported below were carried out with the tapeworm *Calliobothrium verticillatum* (Tetraphyllidea) from the spiral valve of the smooth hound (*Mustelus canis*). The work is necessarily incomplete because of the simultaneous exhaustion of the supplies of fish, isotopically-labeled compounds, and time. Appreciation is expressed to Mr. Robert Lehy and the gentlemen of the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts, for their aid in obtaining infected elasmobranchs.

Received for publication February 16, 1959.

* This study was supported by a grant from the National Institutes of Health, U. S. Public Health Service (E 1508—C1).

** Present address: Department of Biology, The Rice Institute, Houston, Texas.

MATERIALS AND METHODS

Calliobothrium verticillatum was collected from naturally infected *Mustelus canis* trapped in the Woods Hole region. The worms were washed thoroughly in several changes of the salt solution used in previous studies with this worm (Read, Douglas, and Simmons, 1959) containing 300 mM of urea per liter and held in this medium for a period of 60 to 70 minutes at the temperature to be used in the particular experiment (usually 10° C).

The salt solution used in the experiments was modified by the addition of 0.005 M phosphate buffer (pH 7.2) and amino acids at concentrations as indicated in individual experiments. Uniformly labeled L-valine-C^{14} or L-leucine-C^{14} was used in incubation solutions at a level of approximately 0.1 microcurie per milliliter and the corresponding unlabeled amino acid added to obtain the desired chemical concentration. Incubations were carried out in constant temperature water baths, the medium being kept in the bath at the appropriate temperature for 10 to 20 minutes before the experiment. Worms were placed in the experimental medium for the desired period, removed, rinsed by dipping twice in salt solution containing urea, blotted on hard filter paper, and rapidly weighed on a torsion balance. Further washing was unnecessary, since control experiments showed that negligible amounts of activity were removed by a third wash. The worms were then placed in tubes containing exactly 2.0 ml of 70 percent ethyl alcohol. The tubes were immediately stoppered and held for 24 hours at room temperature. During this period the tubes were shaken occasionally. Control studies of the extraction, essentially identical with those of Simmons, Read, and Rothman (1959), showed that complete extraction of "free" isotopically labeled amino acid was obtained by this treatment. For determination of radioactivity, duplicate 0.5 ml portions of each alcoholic extract were plated and counted as described by Simmons, Read, and Rothman (1959).

The data are expressed in counts per minute per gram of wet worm tissue.

RESULTS

When varying quantities of whole *Calliobothrium* were incubated in L-valine-C^{14} or L-leucine-C^{14} for a short interval of time the amounts of amino acid accumulated were found to be directly proportional to the weight of tissue (fig. 1). Thus, samples containing whole worms of various sizes could be used in studying amino acid permeation. When *Calliobothrium* was incubated at low concentrations of L-valine or L-leucine, amino acid permeation followed first order reaction kinetics (fig. 2). When the concentration of the amino acid was varied in incubations of short duration, the permeation fitted an adsorption isotherm (figs. 6 and 7). In the case of L-valine permeation, the apparent dissociation constant (Michaelis constant) is about 2×10^{-3}, and for L-leucine permeation is about 1×10^{-3}.

TABLE I. *The irreversibility of L-leucine permeation.* (All worms incubated in 6×10^{-3}M L-leucine-C^{14} for 2 minutes at 10° C. Controls used to determine the amount of L-leucine permeating, and experimentals incubated for an additional 2-minute period in amino acid-free medium.)

Sample	Counts/minute/gram tissue	
Control (No post incubation)	20,200 22,100 23,100	Av. = 21,800
Experimental (Post-incubation without amino acid)	22,700 20,600 22,200	Av. = 21,700

As might be expected, the time course of amino acid permeation, at concentrations above those yielding a maximum permeation rate, followed zero order reaction kinetics (figs. 3 and 4), the rate of permeation not changing with time. The amino acids can be shown to be accumulated to apparent concentrations exceeding that of the external medium (table III and fig. 3), and the permeation appears to be essentially irreversible (table I). More data are needed to establish this latter point.

The effect of temperature on the penetration of L-valine was determined (figs. 2 and 5). Application of the Arrhenius equation shows that the activation energy (actually heat of activation) is about 9000 calories per mole. The Q_{10} is slightly above 3.

The permeation of L-valine and L-leucine are inhibited by addition of certain, but not all, amino acids of other species (table II). Some experiments on the nature of the inhibitions were carried out and it was shown that the permeation of L-valine is competitively inhibited by L-leucine and that the permeation of L-leucine is competitively inhibited by L-valine (figs. 6 and 7). The apparent dissociation constant for the inhibitor when L-valine is added to inhibit L-leucine permeation is about 2.5×10^{-3} which differs from the apparent dissociation constant for L-valine permeation insignificantly. The apparent dissociation constant for the inhibitor, when L-leucine competitively inhibits L-valine permeation, is about 1×10^{-3} to 2×10^{-3}; this value is only slightly higher than the apparent dissociation constant for L-leucine permeation.

The permeation of L-leucine was inhibited by the addition of iodoacetate or dinitrophenol, if the worms were incubated for 15 minutes in the presence of inhibitor before the addition of the amino acid (table III). In a 2-minute incubation of worms with amino acid plus inhibitor, without previous exposure to the inhibitor, no inhibition of permeation was observed.

The addition of glucose to incubation media at a concentration of 0.01 M was without effect on the velocity of L-leucine or L-valine permeation. The rate of permeation of L-leucine was affected by hydrogen ion concentration, the maximum velocity of permeation occurring at pH 7.4 (fig. 8).

TABLE II. *The effect of other amino acids on the permeation of L-valine and L-leucine in 2 minute incubations at 10° C.* (L-valine or L-leucine present at a concentration of 3.3×10^{-3}M, and each amino acid tested as inhibitor present at concentration of 6.6×10^{-3}M. Values are counts per minute per gram of wet tissue. Figures in parentheses are number of experiments.)

Inhibitor	L-valine permeating	Percent inhibition	L-leucine permeating	Percent inhibition
L-tryptophan	19,625 (4)	0	42,600 (2)	0
L-histidine	21,300 (2)	0	39,600 (2)	0
Glutamic	19,200 (2)	0	44,500 (2)	0
L-arginine	22,000 (2)	0	36,440 (2)	−22
DL-phenylalanine	—	—	37,000 (2)	−20
D-valine	23,000 (2)	0	27,275 (2)	−42
L-isoleucine	19,200 (4)	0	27,670 (2)	−41
L-proline	17,650 (2)	−19	37,150 (2)	−20
Glycine	16,450 (2)	−24	27,450 (2)	−43
N-acetyl-DL-valine	8,500 (2)	−61	—	—
DL-serine	11,500 (2)	−47	18,000 (2)	−61
D-leucine	—	—	14,700 (2)	−69
L-alanine	12,150 (2)	−44	—	—
DL-methionine	8,200 (2)	−63	9,900 (2)	−79
L-cysteine	6,400 (2)	−71	4,150 (2)	−91
L-lysine	26,900 (4)	+20	59,550 (2)	+26
None	21,570 ± 910 (11)		47,066 ± 2100 (10)	

Control experiments were carried out to determine whether the "free" amino acid extracted from the worms was chemically identical with the labeled amino acid added to the medium. Extracts were submitted to paper chromatography and the chromatograms scanned for radioactivity. A tracing from such an experiment is shown in figure 9. The single radioactive component is identical with the material added to the incubation medium.

TABLE III. *The effect of certain inhibitors on the subsequent permeation of L-leucine into* Calliobothrium *in a 2-minute incubation at 10° C.* (Worms preincubated for 15 minutes in the inhibitor before being placed in medium containing the amino acid and the inhibitor.)

Inhibitor	Counts/min/g wet tissue
0	47,780
0	51,500
10^{-4}M Dinitrophenol	23,470
5×10^{-4}M Iodoacetate	21,500
10^{-3}M Iodoacetate	22,100
Medium	11,000*

* Counts per minute per ml.

DISCUSSION

The present study supports the view that an adsorption process is involved in the movement of the amino acids, L-valine and L-leucine, from the external medium into the tissues of *Calliobothrium verticillatum*. Evidences for this are: (1) The non-linear rate-concentration relationship, with first order kinetics at concentrations below a saturation value and zero order kinetics at concentrations above a saturation value; (2) competitive inhibition of amino acid permeation by certain amino acids and inhibitions of as yet undetermined type by a variety of amino acids. Also consistent with such an interpretation are the observations that the rates of permeation of valine and leucine are decreased by certain inhibitors which are not chemical analogs, and that the rate of L-leucine permeation is affected by change in the pH of the medium. The apparent concentration of amino acids against an electrochemical gradient would also be expected to involve an adsorption process. The readiness with which the free amino acid is extracted from the worm's tissues suggests that negligible quantities are chemically bound in the tissues during the very short experimental periods used. However, this cannot be regarded as proven since hydrogen bonds, for example, might be broken by the mild chemical treatment used for extraction. The same caution must be exercised in interpreting the data showing apparent accumulation against an electrochemical gradient.

The data are consistent with the following characterization: The permeation of L-leucine and L-valine obey kinetics expressed by the Michaelis-Menten (1913) equation.

$$1/V = \mathrm{Km}\ 1/\mathrm{Vm}\ S + 1/\mathrm{Vm}$$

where V = rate of permeation, S = concentration of transported material in the external medium, Vm = the maximum rate of permeation, and Km = the Michaelis constant. This equation postulates existence of a chemical complex, the concentration of which determines the maximum rate. Thus,

$$C + S \rightleftarrows CS \rightarrow C + S$$

The equation imposes the condition of a limited number of adsorption sites. As substrate concentration increases, the rate of permeation reaches a maximal value attributable to saturation of adsorption sites with substrate. Higher concentrations of substrate do not increase the rate of permeation. The Michaelis equation is easily tested by plotting 1/V against 1/S. A straight line should be obtained with an intercept equal to 1/Vm and a slope of Km/Vm. Such is the case (figs. 6 and 7).

Thus, we may conclude that we are not dealing with simple diffusion, but with either facilitated diffusion or active transport. Until definite evidence can be

brought forward to show whether the apparent accumulation of L-leucine and L-valine is truly concentration against an electrochemical gradient, we cannot distinguish which of these is involved. It may be pointed out that, from the standpoint of the ecology of the worm, the fact that the amino acids are accumulated is of great significance, regardless of mechanism.

The failure of iodoacetate or dinitrophenol to inhibit amino acid uptake, unless added several minutes before the addition of amino acid, merits some consideration. Other data on the effect of these inhibitors on the energy metabolism of tapeworms has shown that there is a considerable lag after the addition of the compounds before inhibition is observed. It seems probable, therefore, that the amino acids penetrate more rapidly than these inhibitors and that a negligible amount of inhibitor penetrates in the 2-minute experimental period. This may support the concept that the inhibitory effect of these compounds is on energy metabolism rather than directly on the permeation process. It most certainly supports the view that the direct action of these inhibitors is not at the surface. This would merit further study.

There are some interesting differences in the effects of other amino acids on the penetration of L-valine or L-leucine. Surprisingly, D-valine inhibits the uptake of L-leucine, but does not affect the uptake of L-valine at the same concentrations. However, in another experiment (data for which are not presented), D-valine was present at a concentration about 200-fold that of L-valine and a significant inhibition of L-valine uptake was observed. It would seem that the affinity of D-valine for the permeation site of L-valine is lower than its affinity for the site for L-leucine permeation. A similar difference in the effects of L-isoleucine and L-arginine on penetration of L-valine or L-leucine was observed (table 2); L-leucine permeation is inhibited while that of L-valine is not. There are apparent quantitative differences in the effects of glycine, DL-serine and L-cysteine. Further study is required to determine whether or not these are real. Of some interest is a consistent stimulation of L-valine and L-leucine permeation by L-lysine. No explanation is offered to explain these observations: The kinetics of the stimulation should be studied.

The inhibitions of L-valine and L-leucine permeation by the various amino acids studied cannot yet be referred to as competitive. We have shown that L-leucine competitively inhibits the permeation of L-valine and that the latter competitively inhibits permeation of L-leucine. In the case of the other amino acids, we can only conclude that these proved instances of competitive inhibition suggest the probability that all are competitive in nature.

It is unfortunate that Daugherty (1957a, b) and Daugherty and Foster (1958) referred to the inhibition of methionine or cystine uptake by other amino acids as competitive. They presented no evidence that the inhibitions were competitive, and data on relative concentrations of permeating amino acid and inhibitor were not given. Examination of their data suggests that the inhibitors were present at concentrations several hundreds of times those of the penetrating substances studied.

It cannot be concluded from our experiments that all of the amino acids which inhibit the penetration of L-valine and L-leucine will penetrate the worm through similar mechanisms. It is not difficult to visualize the adsorption of an amino acid on a surface permeation site without subsequent permeation occurring. Daugherty and Foster (loc. cit.) concluded that the absorption of alanine, valine, and glycine by *Hymenolepis* must be assumed to take place by "active transfer" because these amino

acids inhibit the penetration of methionine. This assumption is not acceptable without evidence. It may be emphasized that extreme caution should be exercised with respect to conclusions as to the nature of permeation in these and other parasites.

The linear relationship between weight of whole worm samples and the amount of metabolite accumulated in a given time period merits some discussion. It has been shown that in *Hymenolepis* there are differences in the physiology of various portions of the strobila (Read, 1956; Daugherty and Taylor, 1956; Daugherty and Foster, 1958). For this reason, some workers have used anterior portions of worms of given length for experimental material. It has been argued that this eliminates the variation in strobilar physiology. However, it may well be argued that whole animal preparations are preferable. A small differentiated tapeworm is differentiated in the same pattern as a large worm of the same species (assuming there has been no catastrophic destrobilization as is frequently seen after starvation of the host). Our data on the proportionality of tissue quantity and amount of metabolite accumulated would support this view. The use of whole animals may prove to give more reproducible results than the use of standard lengths of strobila. Additional study of this point should be made.

Further discussion of these data will be deferred until publication of more extensive studies on the permeation of amino acids into other tapeworms.

SUMMARY

A study has been made of the kinetics of permeation of L-valine-C^{14} and L-leucine-C^{14} into *Calliobothrium verticillatum*, a cestode from dogfish. The kinetics are those to be expected in a catalytic process. The dissociation constant for L-valine permeation at 10° C is about 2×10^{-3} and for L-leucine is about 1×10^{-3}. Heat of activation for L-valine permeation is about 9000 calories per mole.

Either L-valine or L-leucine competitively inhibits permeation of the other. Certain other amino acids inhibit permeation of L-valine or L-leucine. Permeation of L-leucine is inhibited by previous incubation of the worms in iodoacetate or dinitrophenol. Addition of glucose is without effect on permeation of L-valine or L-leucine. Permeation of L-leucine is affected by pH, maximum permeation occurring at pH 7.4.

LITERATURE CITED

CHRISTENSEN, H. N. 1958 Active transport with special reference to the amino acids. Perspectives in Biol. and Med. **2:** 228–242.

DANIELLI, J. F. 1954 Morphological and molecular aspects of active transport. Symp. Soc. Exp. Biol. **8:** 502–516.

DAUGHERTY, J. W. 1957a Intermediary protein metabolism in helminths. IV. The active absorption of methionine by the cestode, *Hymenolepis diminuta*.. Exp. Parasit. **6:** 60–67.

———1957b The active absorption of certain metabolites by helminths. Am. J. Trop. Med. Hyg. **6:** 464–472.

——— AND FOSTER, W. B. 1958 Comparative studies on amino acid absorption by cestodes. Exp. Parasit. **7:** 99–107.

——— AND TAYLOR, D. 1956 Regional distribution of glycogen in the rat cestode, *Hymenolepis diminuta*. Exp. Parasit. **5:** 376–390.

DAVSON, H. AND DANIELLI, J. F. 1952 The Permeability of Natural Membranes. Cambridge University Press, N. Y.

MICHAELIS, L. AND MENTEN, M. L. 1913 Die Kinetik der Invertinwirkung. Biochem. Ztschr. **49:** 333–369.

PATLACK, C. S. 1956 Contributions to the theory of active transport. Bull. Math. Biophys. **8:** 407–422.

READ, C. P. 1956 Carbohydrate metabolism of *Hymenolepis diminuta*. Exp. Parasit. **5:** 325–344.
———, DOUGLAS, L. T. AND SIMMONS, J. E. 1959 Urea and osmotic properties of tapeworms from elasmobranchs. Exp. Parasit. **8:**
ROSENBERG, T. 1954 The concept and definition of active transport. Symp. Soc. Exp. Biol. **8:** 27–41.
SCHEER, B. T. 1958 Active transport: Definitions and criteria. Bull. Math. Biophys. **20:** 231–244.
SIMMONS, J. E., JR., READ, C. P. AND ROTHMAN, A. H. 1959 Permeation and membrane transport in animal parasites: Permeation of urea into cestodes from elasmobranchs. J. Parasit. In press.
TRIM, A. R. AND ALEXANDER, A. E. 1949 Surface activity and permeability as factors in drug action. Symp. Soc. Exp. Biol. **3:** 111–142.

EXPLANATION OF PLATE I

FIGURE 1. The proportionality of C^{14} labeled amino acid permeation to the weight of worm tissue. Each incubation was for 2 minutes at 10° C with the amino acid at a concentration of 3×10^{-3} M. The difference in the slopes is of no significance since the specific activities of L-leucine and L-valine were different.

FIGURE 2. The permeation of L-valine–C^{14} into *Callibothrium* as a function of time at 0° C and 10° C. L-valine concentration was 7.7×10^{-4} M. a, initial concentration; x, amount permeating the tissues.

FIGURE 3. The permeation of L-valine–C^{14} at a concentration exceeding that yielding a maximum rate. The concentration of L-valine in the medium was 4×10^{-3} M and the permeation rate was about 0.61 micromoles/minute/gram of wet tissue.

FIGURE 4. The permeation of L-leucine-C^{14} at a concentration exceeding that yielding a maximum rate. The concentration of L-leucine was 4×10^{-3} M.

EXPLANATION OF PLATE II

FIGURE 5. The relationship of the velocity (counts per minute per gram wet tissue after 2-minute incubation) to the reciprocal of the temperature in the penetration of L-valine into *Calliobothrium*.

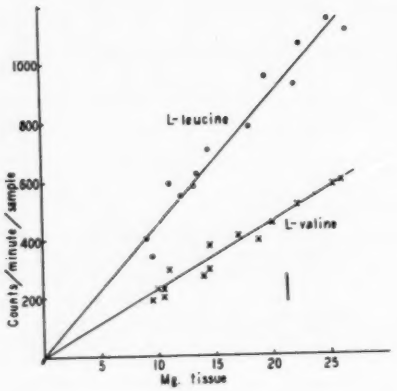
FIGURE 6. The competitive inhibition of L-leucine–C^{14} permeation into *Calliobothrium* by 5×10^{-3} M L-valine. V = counts per minute per gram of wet tissue after 2-minute incubation; S = L-leucine concentration. Curve A, with L-valine; curve B, without L-valine.

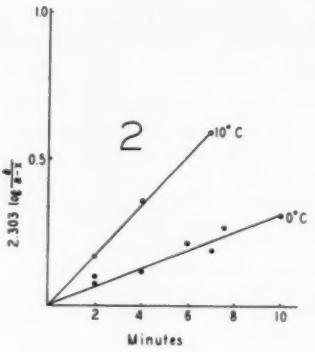
FIGURE 7. The competitive inhibition of L-valine–C^{14} permeation into *Calliobothrium* by 10^{-2} M L-leucine. V and S same as in figure 6. Curve A, with L-leucine; curve B, without L-leucine.

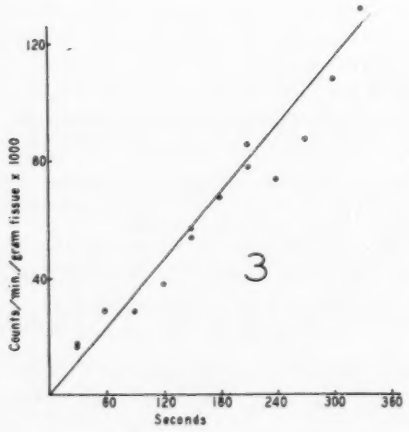
FIGURE 8. The effect of pH on the permeation of L-valine–C^{14} into *Calliobothrium* during a 2-minute incubation. L-valine was at a concentration of 3.5×10^{-3} M in the incubation media. V = counts per minute per gram wet tissue.

FIGURE 9. Scanning of a control chromatogram. The worms were incubated for 5 minutes in the presence of 5×10^{-3} M L-valine–C^{14} and extracted as described in the text. After plate counting, 0.5 ml of extract was spotted and chromatographed on Whatman No. 3 paper using tertiary butyl alcohol, methyl ethyl ketone, ammonium hydroxide and water (10:10:5:3) as the solvent. The strip was then scanned with a Nuclear-Chicago Actigraph II, using a running rate of 12 inches per hour, a 0.25-inch slit width, and an equilibrium time constant of 10 seconds. A radioautograph on Eastman No-Screen X-ray film of this chromatogram showed a single spot corresponding to L-valine. A = origin; B = solvent front. The radioactive "mark" is a spot of C^{14}-labeled ink added for orientation after chromatography. After scanning, the L-valine spot was cut from the paper and eluted with 70 percent alcohol. The eluted sample was plated and counted. It yielded an activity of 540, whereas an identical aliquot of the original extract yielded 619 counts per minute.

PLATE I


L- leucine
L- valine
1
Counts/minute/sample
Mg. tissue


2
10° C
0° C
Minutes


3
Counts/min./gram tissue x 1000
Seconds

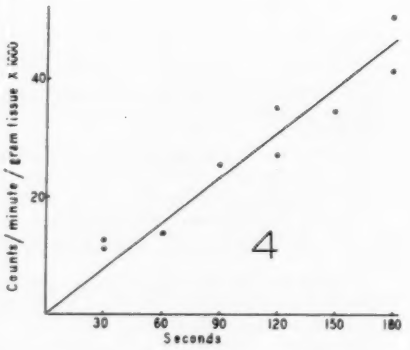
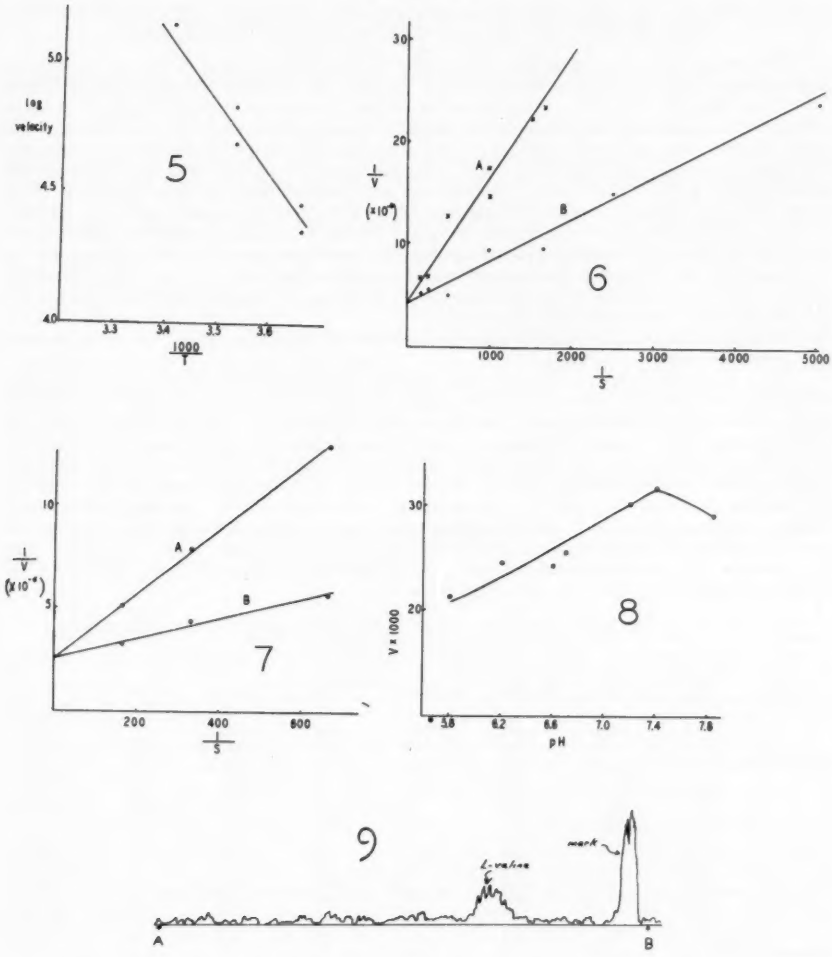

4
Counts/minute/gram tissue X 1000
Seconds

PLATE II



DATES OF MAILING ISSUES OF VOLUME 45 (1959)

No. 1, February, 1959 ..... mailed March 19, 1959
No. 2, April, 1959 ..... mailed April 28, 1959
No. 3, June, 1959 ..... mailed July 17, 1959
No. 4, August, 1959 ..... mailed August 14, 1959
No. 5, October, 1959 ..... mailed October 14, 1959
No. 6, December, 1959 ..... mailed January 18, 1960

RESEARCH NOTE

THE ACTIVITY OF BEPHENIUM EMBONATE AGAINST THE FREE-LIVING AND ADULT STAGES OF *NEMATODIRUS SPATHIGER*.

The efficacy of bephenium embonate (*Franten**) against *Nematodirus battus* and *N. filicollis* was demonstrated by Rawes and Scarnell (1958, Vet. Rec. **70:** 251–255) and confirmed by Gibson (1959, Vet. Rec. **71:** 431–434). At a dosage of 250 mg per kg the drug is 90 percent or more efficient in removing the adult and larval stages of these species. In order to determine the effect of this drug against *N. spathiger* and other gastrointestinal nematodes of sheep, the following controlled test was carried out. Thirteen lambs of mixed white-face breeding and approximately 5 months old were obtained from the breeding flock of this laboratory. The lambs had been naturally exposed to gastrointestinal parasites on irrigated pasture. Six untreated control lambs and 7 treated lambs averaged 70 and 69 pounds, respectively, before treatment. Lambs were individually weighed and each of the treated group received 250 mg per kg of an aqueous suspension of bephenium embonate by mouth using a dose syringe with a Whitlock nozzle. Five days after receiving the drug all the lambs were weighed, killed by electrocution and the intestinal tracts examined for worms.

No toxicosis was observed following administration of the drug, and no significant changes in weight occurred.

Table I lists the results of the host examinations. All figures refer to mature worms since no attempt was made to harvest and count preadult stages. Bephenium embonate was inactive against *Ostertagia circumcincta* and *Trichostrongylus colubriformis*. The numbers of *Haemonchus contortus* and *T. axei* are rather low, and although there was no statistical difference between treated and untreated groups at the 5 percent level of confidence, it appears that there was some removal of these species. The 77 percent efficacy of bephenium embonate in removing *Marshallagia marshalli* was found to be statistically significant at the 1 percent level of confidence. Complete absence of *N. filicollis* in the treated lambs indicates a high level of activity of the drug despite the relatively small numbers of worms in the untreated lambs. It is clear that *N. spathiger* was effectively removed, since an average of 37 worms remained in the treated lambs as compared to 2007 in the untreated lambs.

TABLE I. *Worm counts in treated and untreated lambs and percentage efficiency of bephenium embonate.*

Group	Ear Tag	*H. con.*	*O. circ.*	*M. marsh.*	*T. axei*	*N. spath.*	*N. fili.*	*T. col.*
Untreated	573	240	2300	200	460	1920	0	4000
	617	120	1020	480	240	1240	300	2780
	664	0	640	320	0	1900	0	5580
	633	300	260	1800	0	1050	110	18840
	667	0	400	960	0	3480	0	5000
	584	140	500	80	180	2450	350	6860
Average		133	853	640	147	2007	127	7177
Treated	661	0	1140	0	0	0	0	7220
	632	0	400	320	80	0	0	5280
	614	0	410	150	0	0	0	6160
	666	60	240	0	120	140	0	5460
	624	160	820	60	60	120	0	5080
	658	0	380	500	0	0	0	5660
	585	120	1680	0	0	0	0	5760
Average		49	724	147	37	37	0	5803
Percentage removal		63	15	77	75	98	100	19

H. con., Haemonchus contortus; O. circ., Ostertagia circumcincta; M. marsh., Marshallagia marshalli; T. axei, Trichostrongylus axei; N. spath., Nematodirus spathiger; N. fili., N. filicollis; T. col., Trichostrongylus colubriformis.

Experiments were carried out to determine whether bephenium embonate had any effect on the free-living stages of *N. spathiger*. Eggs of *N. spathiger* (Strain KT) were completely freed of fecal debris and allowed to incubate in water at 30° C with various concentrations of the drug. Even in a saturated solution where there was an excess of the compound, embryonation proceeded at a normal rate, the larvae hatched and were unharmed after 8 days additional contact.

The assistance of Dr. C. J. Mode, Department of Mathematics, Montana State College, in statistical analysis of the data is gratefully acknowledged.

This is a contribution from the Montana Veterinary Research Laboratory (Montana Agricultural Experiment Station and Montana Livestock Sanitary Board cooperating), Agricultural Experiment Station, Montana State College, Bozeman. Paper No. 468, journal series.—W. C. MARQUARDT, D. H. FRITTS, N. R. MCALPIN AND W. W. HAWKINS, JR., *Montana Veterinary Research Laboratory, Montana State College, Bozeman.*

* Burroughs Wellcome and Company who also supplied the drug for this test.

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: PERMEATION OF UREA INTO CESTODES FROM ELASMOBRANCHS*

J. E. Simmons, Jr., C. P. Read and A. H. Rothman

Marine Biological Laboratory, Woods Hole, Massachusetts, and Department of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

Recently, Read, Douglas and Simmons (1959) reported certain effects of urea on some cestodes of the order Tetraphyllidea. They found that when removed directly from the hosts, *Callibothrium verticillatum* and *Phyllobothrium foliatum* contain high concentrations of urea, averaging 3.7 percent of the dry weight in the case of *Calliobothrium*. They presented evidence that urea is an osmotically active component of the tissues of these worms. In salt solutions not containing urea, the worms undergo an initial hydration of large magnitude, subsequent volume regulation being accompanied by a loss of urea from the tissues. Addition of 200 mM of urea per liter of salt solution resulted in a great, though not total, diminution of the initial hydration, and the return of the worms to the approximate original weight was more rapid.

The present studies are an examination of the kinetic aspects of urea permeation and exchange in some Tetraphyllidea.

MATERIALS AND METHODS

The cestodes used in this study were obtained from hosts trapped in Buzzard's Bay, Massachusetts. Collection and handling of worms was done as previously described (Read, Douglas, and Simmons, 1959). Cestodes studied were the tetraphyllid species, *Calliobothrium verticillatum* from the smooth dogfish, *Mustelus canis*, and *Onchobothrium pseudo-uncinatum* from the northern sting ray, *Dasyatis centrura*.

Incubations of worms were carried out in open vessels in controlled temperature water baths. Worms were incubated as individual samples in vials containing 2 ml of medium or, in some experiments, were incubated in a larger volume (30 to 50 ml) in a single vessel, worm samples being withdrawn at appropriate intervals.

Incubation solutions contained salts in concentrations used in earlier experiments (Read, Douglas, and Simmons, 1959). These were modified by the addition of 0.005 M phosphate buffer (pH 7.2). Unless otherwise stated, urea was added to the solution at a concentration of 300 mM per l when used with tapeworms from the dogfish and 400 mM per l with those from the ray. In solutions containing C^{14}-urea, the isotope concentration was about 0.1 microcurie per ml, the total urea content of such solutions being adjusted to the desired concentrations.

Worm samples (20 to 60 mg of wet tissue) removed from solutions containing C^{14}-urea were rinsed twice by dipping in about 25 ml portions of the test solution lacking labeled material, blotted on hard filter paper, and rapidly weighed on a torsion balance. They were held for analysis in individual test tubes containing 2.0 ml 70 percent ethanol. Samples of media were made to a convenient dilution with ethanol in volumetric flasks.

It was determined that urea contained in the worm was extracted by the alcoholic supernatant. In a control experiment, the worms were allowed to stand overnight, a sample of the alcohol extract was removed for C^{14} analysis, and the remainder of the extract was discarded. The worms were washed once with 70 percent ethanol and homogenized in 4 ml of fresh alcohol. This suspension was centrifuged sharply and the supernatant fluid tested for C^{14}. No appreciable radioactivity was present. Thus, analysis of the radioactivity of the original alcohol in which the worms were held was considered to be a measure of the radioactivity present in the worms.

Received for publication February 16, 1959.

* This work was supported by a grant from the National Institutes of Health, U. S. Public Health Service (E-1508).

For counting, 0.5 ml aliquots of the alcoholic supernatant were pipetted on aluminum planchets (31 mm diameter × 2 mm deep) and evaporated to dryness under an infra-red lamp. Prior to introducing a sample, each planchet bottom was ringed at the periphery with wax. This, and handling the preparations on a leveling table, served to restrict samples to a uniform surface area as they dried. These precautions, together with constant geometry of counting, precluded the necessity of applying any correction for geometry. Each worm sample was prepared and counted in duplicate; 118 pairs of duplicates gave a mean difference of count of 4.6 percent. Samples were counted with a Nuclear-Chicago model D47 gas-flow counter, using a Nuclear-Chicago model 181-A decade scaler for recording. A sufficient number of counts were taken in all cases to insure a counting error of less than 5 percent; in the great majority of cases less than 3 percent counting error would be expected. Background counts were taken before and after each 3 sets of duplicate samples, with a blank planchet in place. During the course of this work, 755 minutes of background counts were taken; the mean background was 27.7 ± 0.4 counts/minute.

For urea analyses, appropriate aliquots of the alcoholic supernatants from worm and gut samples, or of sera, were evaporated to dryness at approximately 100° C, redissolved in 0.5 ml 0.05 *M* phosphate buffer (pH 6.8), and allowed to react with 0.02 ml urease solution for 30 minutes at room temperature. The urease solution was a filtered 4 percent solution in saturated NaCl prepared from the commercial product (Arlington). The ammonia liberated was collected by the microdiffusion method of Seligson and Seligson (1951). The ammonia captured was determined by the procedure of Lang (1958). All samples were determined in duplicate and corrected for urease blanks and tissue blanks carried through the procedure with each sample. Urea recoveries averaged 101 percent (93–109).

Other experimental procedures used will be mentioned in context.

EXPERIMENTAL

Calliobothrium verticillatum from a single dogfish captured the day before the experiment were divided into 2 groups. One group was incubated for 1 hour at 19.5° C, in the standard solution lacking urea in order to deplete these worms of endogenous urea. The other group was similarly incubated in salt solution containing urea (300 mM/l) for the same period.

Urea analyses of a sample of worms removed directly from the dogfish showed a urea content of 1.10 percent of the wet weight. Analyses of worm samples from the 2 groups above, after 1 hour showed urea values of 1.05 percent in the case of those incubated in the presence of urea, 0.14 percent for those incubated in urea-free solution.

After this period, both experimental groups were removed, blotted, and transferred to salt solution containing C^{14}-urea; samples from both groups were removed for analysis after various intervals. Results of these analyses are presented in figure 1.

It is apparent that the rate of exchange between urea in the medium and that in the worms is very high; indeed, it is comparable to the rate of permeation into the worms depleted of urea. In both groups, the rate of entry of urea is most rapid initially and diminishes with time. It may, however, be concluded from these data that the concentration of urea in the tissues of worms previously depleted of urea has not yet reached equilibrium with that of the medium after 1 hour. Moreover, it is also apparent that the isotopic urea in those worms not depleted of urea has also not equilibrated in 60 minutes with the urea in external solution. It is apparent that the specific activities (counts/minute/mg urea) of urea entering the depleted worms, remains the same as that of the medium, indicating that the urea molecule in the tissues of the worm is present in an unaltered state.

In further experiments, unless otherwise stated, prior depletion of endogenous urea of the worms by incubation in standard salt solution lacking urea for 1 hour was adopted as a standard procedure.

In order to determine the time required for equilibration of urea in the worm with that of the external environment at a known temperature, *Calliobothrium* was incubated at 20° C for longer periods in solutions containing C^{14}-urea. These data are given in figure 2. It may be seen that equilibration is essentially effected in the 60 to 90 minute interval, under these conditions.

To determine the effects of temperature on urea permeation, *Calliobothrium* was incubated for various intervals in a C^{14}-urea solution at 0°, 10°, and 21° C. Graphically, the data represent a family of curves (fig. 3); these will be discussed further below.

The effect of urea concentration on permeation and exchange was studied, using worms preincubated with and without urea. The data are presented in figure 4. It is again seen that exchange is comparable to the degree of entry of this substance into depleted worms. These results also substantiate the previous conclusion (Read, Douglas, and Simmons, 1959), derived from chemical analyses, that the urea content of *Calliobothrium* is a direct function of the concentration of this compound in the external environment. This relationship is essentially linear when the concentration of urea in the medium is 200–400 mM/1, but diminishes in the 100–200 mM/1 range. This reflection of a change in the rates of urea permeation and exchange is not readily explainable. It is probably related to osmotic effects, but further work would be required to clarify this point.

Since previous studies indicated that urea readily leaked from *Calliobothrium* (Read, Douglas, and Simmons, 1959), the rate of urea leakage was determined by incubating worms in a C^{14}-urea solution for 2 minutes, briefly rinsing them, transferring them to a similar solution lacking C^{14}-urea, and withdrawing worm samples at appropriate intervals. Zero-time samples were those incubated in the labeled solution and rinsed, but not incubated in label-free solution. Such data are plotted in figure 5; for comparison, data on the uptake of C^{14}-urea are also figured. It is apparent that the rate of leakage of urea from *Calliobothrium* closely approximates the rate of entry of this compound.

Read, Douglas, and Simmons (1959) reported that *Phyllobothrium foliatum* and *Inermiphyllidium pulvinatum,* from *Dasyatis centrura,* resemble *Calliobothrium* in that weight changes in salt solutions are modified similarly by the addition of urea. These worms were unavailable during the present study; however, a single experiment was done with another tetraphyllid, *Onchobothrium pseudo-uncinatum,* from *D. centrura.* The uptake of C^{14}-urea by *Onchobothrium* was determined at 20° C and is shown in figure 6. Comparison of these data with those obtained with *Calliobothrium* indicates that urea entry occurs similarly but at a markedly lower rate in *Onchobothrium.*

Addition of glucose to a concentration of 0.01 M or variation of pH between 6.0 and 8.0 had no effect on the rate at which C^{14}-urea penetrated *Calliobothrium.*

DISCUSSION

The most important single criterion for establishing that permeation occurs by an active mechanism is the demonstration that accumulation of a substance occurs against a concentration gradient.

Until accurate density measurements (or volume determinations) of *Calliobothrium* are available, allowing direct comparison of equivalent concentrations of urea inside and outside the worm, it cannot be categorically stated that accumula-

tion does not occur. However, the data in the present report provide such strong evidence for a non-catalytic mechanism of urea permeation, that lack of information regarding the above-mentioned parameter does not assume critical importance; indeed it can be inferred that accumulation does not occur.

It is probably significant that changes in pH do not affect urea permeation, since pH effects have been observed on permeation of materials into cestodes for which there is evidence that active transport or facilitated diffusion mechanisms are involved. (Read, Simmons, and Rothman, 1959; Phifer, 1959.)

Results obtained in the present investigation corroborate previous observations that the concentration of urea within the worm is a direct linear function of its concentration in the external environment in the range of urea concentrations studied. Such an effect would be expected if urea enters by simple diffusion, and would not be expected in a catalytic system. It should be pointed out that the highest concentration of urea used (400 mM/l) is considerably greater than the physiological level usually observed in the host. This fact makes it seem more likely that the observed effect of external concentration of urea on its permeation into *Calliobothrium* is a result of simple diffusion.

Certain inferences may be drawn from the effects of temperature on urea permeation in *Calliobothrium*. First, as mentioned above, the permeation rate at a given temperature diminishes with time, implying that the permeation rate is not independent of concentration. These data would, of course, be markedly influenced if urea were metabolized or if the substance is in a bound state within the worm. The fact that equilibrium of urea inside and that outside the worms is so readily reached and that urea levels in the worm following incubation in approximate isosmotic concentrations of the substance so closely approach the values of freshly collected worms argues strongly that metabolic alteration of urea does not occur. That urea readily leaks and is so easily extracted from the worm tissues would indicate that, if urea is bound in the tissues, the bonding is very weak.

Diffusion in liquid systems, of model membranes and in living cells has been found to be concentration-dependent and has been described by equations denoting kinetics of the first order (Davson and Danielli, 1952). Our data obtained from the experiment on the temperature effect on urea permeation were analyzed to determine whether first-order kinetics might apply. Figure 7 shows a plot of log Co/C versus time (symbols defined in the figure). It is apparent that a simple linear relationship does not exist. In the figure, the data for each curve are interpreted as a linear function with a break at about 10 minutes; they could also be interpreted as a curvilinear function with a marked change in slope. It must be said that any interpretation of these data is necessarily speculative. However, we feel that certain possibilities may be briefly discussed. Permeation of a substance into an organism such as a cestode, even if occurring by simple diffusion, will most assuredly be more complicated of analysis than classical studies of diffusion in liquids. It will undoubtedly also be more complex than diffusion through model membranes or into certain single cell preparations. In the latter case, some justification can be made for treating the object under study as a homogeneous mass with a single barrier separating external environment and internal structure; no such situation exists with cestodes. The complex internal organization precludes its being considered homogeneous, and it is almost certain that more than 1 barrier to permeation of an

external substance exists. More specifically, such a substance would first have to pass through the "skin" of the cestode, and to become intracellular must also traverse the cell membrane. Hence, at least 2 barriers, and probably more, will be encountered. Such barriers may differ in permeation characteristics or even in mechanism involved in penetration. The result would be the existence of "compartments" into which a substance must enter. Moreover, from previous work (Read, Douglas, and Simmons, 1959) it is apparent that water shifts as well as shifts of other materials occur when *Calliobothrium* is transferred between solutions containing or lacking urea. These result in area-volume changes which may be of varying magnitude as they apply to "compartments" within the worm. It would seem that accurate measurements of such area and volume changes of such compartments will be technically difficult to obtain, especially coincidental to other measurements.

Wilde (1955) feels that when multiple compartments exist, mathematical analysis may be impossible if the sequence of change from one to another is unknown. Robertson (1957) has given theoretical analyses of systems involving compartition, and the difficulties to be encountered in the study of such situations are clearly indicated. The present authors feel that permeation of materials into cestodes presents problems of sufficient complexity, that extreme caution must be exercised in analysis. Hence, figure 8 probably represents a simplified analysis; actually, complications such as those mentioned above probably are superimposed on a simple pattern. However, since linearity is closely approached for the first 10 minute time increment, velocity coefficients were calculated, using the usual formulae for first-order chemical reactions. These proved to be 2.7×10^{-3} sec^{-1}, 4.2×10^{-3} sec^{-1} and 5.8×10^{-3} sec^{-1} for respectively 0° C, 10° C, and 21° C. Thus, the calculated Q_{10} over the interval 0° to 10° C is 1.5 and from 10° to 21° C, $Q_{10} = 1.4$. It should be emphasized that velocity coefficients for diffusion into living cells are also a function of the ratio area/volume (Davson and Danielli, 1952), which is not easily determined for cestode materials. This ratio, however, will be less than unity and consequently the estimated Q_{10} values are probably high.

There is no indication from any of the data obtained in the present study that catalysis is involved in urea permeation in *Calliobothrium*. That such permeation occurs by simple diffusion is indicated by (1) linearity of the amount permeating with external concentration, (2) a leakage rate comparable to rate of entry, and (3) the numerical values of Q_{10} obtained.

SUMMARY

C^{14}-urea was used to study various factors involved in urea permeation of certain tetraphyllidean cestodes of Elasmobranchs. The rate of urea permeation of *Calliobothrium verticillatum* decreases with time, and urea inside the worm apparently comes to equilibrium with that in the medium during a 60 to 90 minute incubation period. The rate of exchange between worm and medium closely approximates the rate of entry into the worm. The rate of leakage of urea from *Calliobothrium* is comparable to the rate of uptake. Urea in the worm is apparently a direct linear function of urea concentration in the external environment. Alteration of pH or addition of glucose do not affect the rate of permeation. Estimated Q_{10} values of urea permeation in *Calliobothrium* are 1.5 for 0° to 10° C and 1.4

for 10° to 21° C. There is no evidence that urea permeation involves catalysis. Rather, simple diffusion is indicated as the mechanism of permeation. A single experiment with *Onchobothrium pseudo-uncinatum* revealed a much lower rate of urea uptake than with *Calliobothrium*.

LITERATURE CITED

DAVSON, H. AND DANIELLI, J. F. 1952 The Permeability of Natural Membranes. Cambridge University Press.

LANG, C. A. 1958 Simple microdetermination of Kjeldahl nitrogen in biological materials. Anal. Chem. **30:** 1692–1694.

READ, C. P., DOUGLAS, L. T. AND SIMMONS, J. E. 1959 The relation of urea to osmotic properties of some Tetraphyllidean cestodes. Exp. Parasit. In press.

READ, C. P., SIMMONS, J. E. AND ROTHMAN, A. H. 1959 Permeation and membrane transport in animal parasites: Amino acid permeation into tapeworms from elasmobranchs. J. Parasit. In press.

PHIFER, K. O. 1959 Permeation and membrane transport in animal parasites: The absorption of glucose by *Hymenolepis diminuta*. J. Parasit. In press.

ROBERTSON, J. S. 1957 Theory and use of tracers in determining transfer rates in biological systems. Physiol. Rev. **37:** 133–154.

SELIGSON, D. AND SELIGSON, H. 1951 A microdiffusion method for the determination of nitrogen liberated as ammonia. J. Lab. Clin. Med. **38:** 324–330.

WILDE, W. S. 1955 Transport through biological membranes. Ann. Rev. Physiol. **17:** 17–36.

RESEARCH NOTE

ON THE LIFE CYCLE OF *NEODIPLOSTOMUM BUTEONIS* AND THE OCCURRENCE OF A PERIPROSTATE (TREMATODA: DIPLOSTOMATIDAE).

Neodiplostomum buteonis Dubois and Rausch, 1950, was found in one snowy owl, *Nyctea scandiaca*, one great horned owl, *Bubo virginianus*, and two barred owls, *Strix varia*, taken in southern Ontario. Eggs from the intestinal contents of the snowy owl were used in studying the life cycle. One of 24 *Physa* sp. exposed to infection subsequently released cercariae. The cercaria was similar to that of *Neodiplostomum texensis* described by Chandler (1942, Tr. Am. Micr. Soc. **61:** 156–167), with 2 pairs of pre-acetabular penetration glands, 1 pair of caudal flame cells, and no pre-oral spines. Cercariae penetrated tadpoles of *Rana clamitans* and *Rana sylvatica* and developed into diplostomula free in the body cavity. The diplostomulum had a well developed reserve excretory system with cocciform to bacilliform excretory granules in terminal blind pouches and a holdfast shorter than the diameter of the ventral sucker. Diplostomula were fed to 2 laboratory-reared garter snakes, *Thamnopis sirtalis*. One snake, examined a month later, had unencapsulated diplostomula in the oesophageal and stomach walls, pericardium, and pancreas; and the other snake, examined 1 year later, had encapsulated diplostomula in the fat body and intercostal muscles.

Sagittal sections of the adult revealed the presence of a distinct periprostate about the ejaculatory duct, a finding of possible phylogenetic significance as the presence of a prostate gland (either paraprostate or periprostate) is a primitive feature common to most proterodiplostomatids, but known at present from only one other diplostomatid, *Podospathalium pedatum*. (See Dubois, G., 1938, Mém. Soc. Neuchât. Sci. Nat. VI).

I should like to thank Dr. H. B. Speakman, formerly Director, Ontario Research Foundation, for providing facilities to do this work.—J. C. PEARSON, *University of Queensland Veterinary School*.

PLATE I

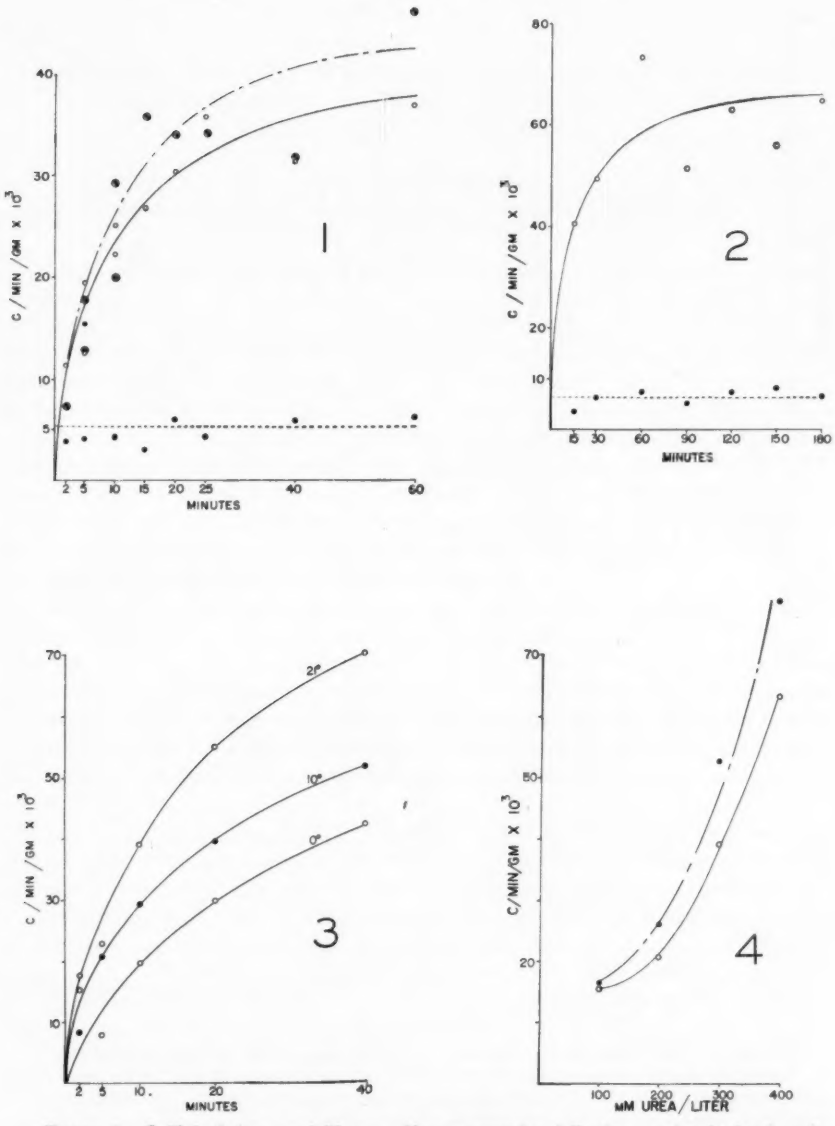


FIGURE 1. *Calliobothrium verticillatum.* Urea permeation following pre-incubation in solutions with and without urea. Activities of urea in worms are reported as counts per minute per gram wet worm tissue, corrected for background. Specific activities of urea in worms or in the medium are counts per minute per milligram urea, corrected for background. Checkered circles = activities in worms pre-incubated with urea. Open circles = activities in worms pre-incubated without urea. Solid circles = specific activities of urea in worms pre-incubated without urea. Dotted line represents specific activity of the urea in the medium.

FIGURE 2. *Calliobothrium verticillatum.* Urea permeation during a 3-hour experimental period. Symbols as in figure 1.

PLATE II

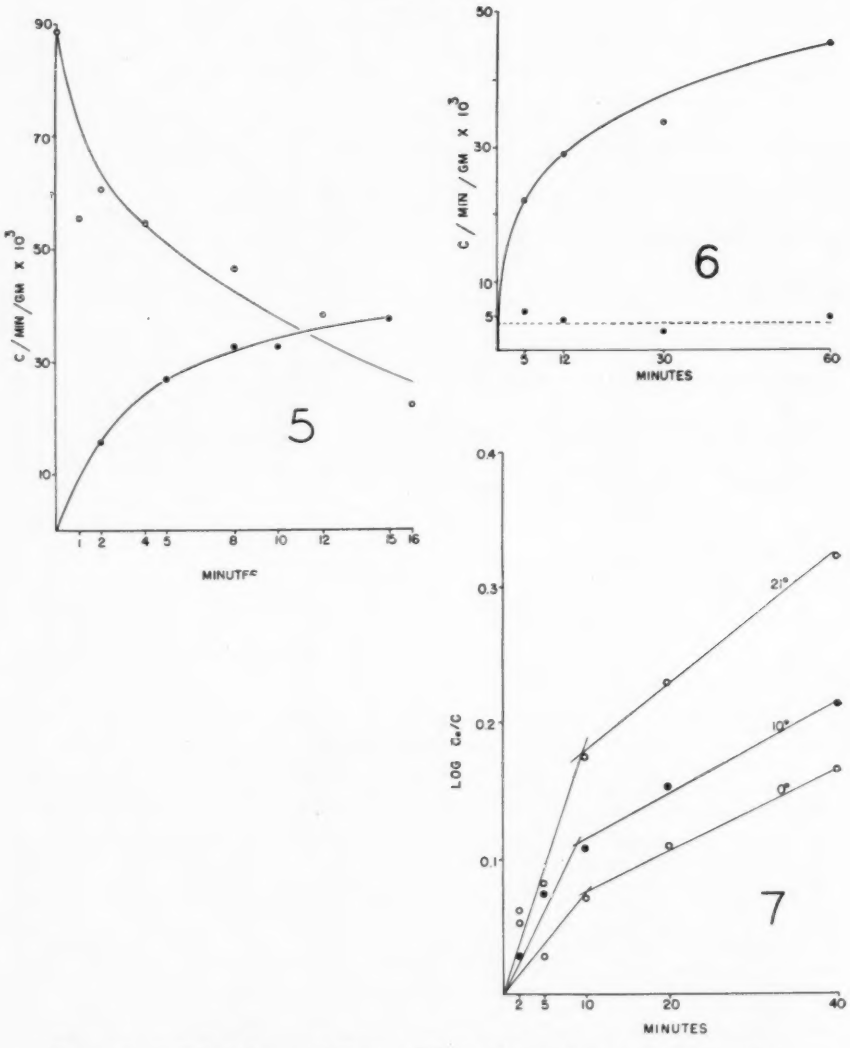


FIGURE 3. *Calliobothrium verticillatum.* Effect of temperature on urea permeation.

FIGURE 4. *Calliobothrium verticillatum.* Effect of concentration of urea in the medium on urea permeation. Open circles = activities in worms pre-incubated without urea. Solid circles = activities in worms pre-incubated with urea. Temperature = 20° C.

FIGURE 5. *Calliobothrium verticillatum.* Urea leakage (compared with urea permeation). Temperature = 20° C.

FIGURE 6. *Onchobothrium pseudo-uncinatum.* Urea permeation at 20° C. Symbols as in figure 1.

FIGURE 7. *Calliobothrium verticillatum.* Analysis of temperature effect on urea permeation. C_o = initial concentration of urea in medium. C = concentration of urea in medium remaining at each time interval, calculated from amount taken up per gram wet worm tissue.

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: THE ABSORPTION OF GLUCOSE BY *HYMENOLEPIS DIMINUTA**

KENNETH PHIFER**

Department of Pathobiology, School of Hygiene and Public Health,
Johns Hopkins University, Baltimore, Maryland

Understanding the physiology of tapeworms requires the elucidation of processes of nutrition. Since the tapeworm lacks an alimentary tract of any kind, the only means for entry for food is through the integument. Bathed in a medium of complex composition in the intestine, the tapeworm is directly dependent on processes involving penetration of materials from the environment. Read, Simmons and Rothman (1959) have shown that, in the movement of certain amino acids across the membranes of the tapeworm *Calliobothrium* from marine elasmobranch fishes, diffusion definitely does not account for the observed phenomena. Read (1955) demonstrated, by electron microscopy, villus-like structures on the surface of tapeworms and suggested that micro-canals observed might be associated with absorptive function. These structures suggest that the integument may be an active organ containing many organelles functional in absorption.

Much work has been published dealing with the uptake of glucose by red blood cells, isolated loops of intestine, and certain other cells or tissues of vertebrates. The conclusions in each case are generally that the process of diffusion cannot alone account for the results obtained. Various theories have been presented as to the mechanism. In some cases, the energy derived from the metabolic processes probably is required for the uptake of glucose by the tissue to occur; while, in other cases, little work is performed and negligible amounts of metabolic energy seem to be required.

In determining whether glucose permeation occurs by diffusion or by a catalytic process (either active transport or facilitated diffusion) rigorous experimental analysis is necessary. The criteria appropriate to such an analysis have been reviewed by Read et al (1959). In the experiments, the results of which are reported in this paper, an attempt was made to ascertain the nature of glucose permeation in a tapeworm. The effects of potassium ion concentration, the relative uptake rates at various stages in the development of the worm, and the effect of worm size on the rate of uptake of glucose have also been studied.

METHODS

Sprague-Dawley rats were used to maintain *Hymenolepis diminuta* infections. Each rat was infected with 10 cysticercoids unless otherwise noted. The worms were then allowed to grow to the definitive "adult" size (ca 20 days).

Received for publication February 16, 1959.

* The author wishes to express gratitude to Dr. C. P. Read for his guidance during the course of this study. The work was supported in part by grants from the National Institutes of Health, U. S. Public Health Service (E-1508).

** Public Health Service Research Fellow of the National Institute of Allergy and Infectious Diseases. Present address: National Institutes of Health, P. O. Box 717, Columbia, S. C.

Two different experimental approaches were utilized in the present study. Incubations were carried out for periods of 30 minutes and chemical analysis carried out to determine the disappearance of glucose from the suspending medium; such experiments are referred to as extended time experiments. A quite different approach involved the use of isotopically labeled glucose. The worms were incubated for 60 seconds and the amount of labeled compound permeating the worms was extracted at termination of the incubation; such experiments are referred to as short interval experiments.

Infected rats were starved 24 hours before being killed by a blow on the head. A fasting period has been found to significantly increase glucose utilization by this tapeworm (Read and Rothman, 1957a). The worms were removed from the intestine of the host and washed in Krebs-Ringer solution, buffered at pH 7.5 with 0.03 M trishydroxy-aminomethane (Tris). This buffer was preferred over phosphate since it was thought that phosphate might have an effect upon the glucose uptake rate.

In extended time experiments, the worms were then put into flasks in a 37° C water bath and were shaken slowly for 45 minutes. At the end of this period, the worms were transferred to the reaction vessels, one worm to each 25 ml Erlenmeyer flask containing 5 ml of glucose solution. After a preliminary study of the effect of glucose concentration on the uptake rate, the concentration of 8.3×10^{-3} M was determined to be the most suitable from a kinetic standpoint.

The flasks were stoppered, placed in a water bath and allowed to shake slowly for 30 minutes. Unless otherwise stated, all incubations were at 37° C. At the end of the incubation period, the worms were removed from the flasks, placed in tares, and put into an oven set at 70° C. They were removed 24 hours later and were weighed. One ml aliquots from each incubation flask were pipetted into tubes containing 7 ml H_2O. To each tube was added 1 ml 10 percent $ZnSO_4$ and 1 ml 0.5 N NaOH. The tubes were centrifuged, and 1 ml was taken from the supernate of each tube. These 1 ml samples and a set of glucose standards were analyzed by the procedure of Nelson (1944) to determine the amount of glucose remaining in the flasks after incubation. A flask containing glucose but no worm was run with each set of experimental flasks. When the dry weights of the worms were determined, the uptake per mg of dry weight of worm tissue per hour was calculated. This figure is expressed throughout in micrograms of glucose.

In short interval experiments, experimental worms were from hosts which had not been starved. Worms were removed from the rats and treated as indicated above except that the pre-incubation period in Krebs-Ringer solution was 1 hour rather than 45 minutes. After this pre-incubation period single worms were added to a 20 ml beaker containing approximately 0.1 microcuries of labeled glucose per ml. In most experiments the concentration of glucose varied from 1 to 5×10^{-3} M and the incubation time was 60 seconds unless otherwise stipulated. After 60-second incubation, each worm was rinsed in two 100 ml portions of Krebs-Ringer solution, blotted on filter paper, and placed in a tube containing 5 ml of 50 percent alcohol. The tubes were agitated on a shaking table for 1 hour and were allowed to stand at room temperature overnight. The tubes were then shaken and 2 samples of 0.5 ml from each tube were plated and counted by the procedure described by Simmons, Read, and Rothman (1959). It was found that 50 percent alcohol-extracted dry weight does not differ significantly from "normal" dry weight; therefore, the data are expressed in terms of dry weight, the worms being dried as described above. In most cases the rates are expressed in counts per minute, since relative values are more important than absolute ones.

RESULTS

The effects of the incubation temperatures, 15°, 25°, 35°, 37°, and 40° C were studied in extended time experiments. As can be seen from table I, the effect of incubation temperature on the rate of glucose uptake by *H. diminuta* differs considerably from one temperature range to another. In the range of temperatures approximating that of the rat intestine, the relative increase is much greater. The Q_{10}'s for temperature intervals of less than 10° were calculated using the formula, $\log Q_{10} = \frac{10}{t_2 - t_1} \times \log \frac{k_2}{k_1}$, k in this case being the uptake rate at a particular temperature. The results in table I are based on 3 experiments at each temperature, using 15 worms for each experiment. In these experiments, the temperature of the bath did not vary from the desired temperature by more than 0.05° C. Figure 1 shows an Arrhenius plot of the data, K being simply the uptake rate. The slope of the curve

TABLE I. *The influence of temperature on the uptake of glucose by* H. diminuta *at pH 7.5 in extended time experiments.*

Experiment	Temperature	Number of worms	Uptake rate and S.D.	Mean rate	Temperature range	Q_{10}
1	15°	14	10.1 (4.1)			
2	"	"	11.1 (3.2)	10.0		
3	"	"	8.7 (5.9)			
4	25°	14	25.8 (9.0)			
5	"	"	26.5 (10.2)	28.3	15°–25°	2.83
6	"	"	32.5 (18.4)			
7	35°	14	70.8 (10.3)			
8	"	10	77.7 (13.4)	78.0	25°–35°	2.76
9	"	14	85.5 (7.7)			
10	37°	14	85.2 (13.1)			
11	"	"	113.7 (11.7)	100.6	35°–37°	3.46
12	"	"	102.9 (7.3)			
13	40°	14	129.4 (12.9)			
14	"	"	143.3 (8.4)	140.0	37°–40°	3.12
15	"	"	147.0 (16.1)			

The uptake rates are expressed in terms of micrograms of glucose taken up per milligram of dry weight of worm tissue per hour. Q_{10} values are based on differences between mean rates of uptake.

was found to be 0.00458, and an activation energy (= heat of activation) of 20,910 calories was calculated from these data.

The effect of temperature was also examined in short interval experiments, i.e., by extracting glucose from the worm after a 60-second incubation in labeled glucose (5×10^{-3} M). From table II one can see that the relative effects of temperature agree with those found using the indirect method. The slope of the plot shown in figure 2 was found to be 0.005 and the calculated energy was 22,800 calories.

Magee and Reid (1931), attempting to show that glucose is transformed into a phosphate compound during absorption, were the first to demonstrate an effect of pH on the absorption of glucose. They showed that the addition of phosphate buffer at pH 7.0 increased the rate of glucose absorption by rat intestine. It was later shown by Laszt (1935), using other buffers, that the accelerating action of the

TABLE II. *The influence of temperature on the uptake of glucose by* H. diminuta *at pH 7.5 during short interval incubations.*

Temperature	Uptake rate	Mean rate	Temperature range	Q_{10}
	16.4			
	13.4			
	6.7			
7°	6.2	10.1		
	9.1			
	8.7			
	24.6			
	23.8			
	25.1			
17°	17.8	24.5	7°–17°	2.43
	21.3			
	20.6			
	35.2			
	28.0			
	76.9			
	87.2			
	99.5			
	78.5			
27°	64.3	87.9	17°–27°	3.59
	125.0			
	88.8			
	68.9			
	85.7			
	342.7			
	363.5			
	302.5			
37°	258.5	283.0	27°–37°	3.22
	229.4			
	289.0			
	197.2			

The uptake rates are expressed in terms of counts per minute per mg. of dry weight of worm tissue. Q_{10} values are based on differences between mean rates of uptake.

phosphate was due to its activity as a buffer.

With this in mind, extended time experiments were carried out to test the effect of pH on the absorption of glucose by the tapeworm. Electrometric determinations were made before and after the incubations to be certain that the hydrogen ion concentration remained constant. The data summarized in figure 3 show that the pH optimum is about 7.6 with a slight secondary peak at pH 8.5. (These data were obtained by incubating worms for 30 minutes followed by an examination of the medium for glucose.)

TABLE III. *The effect of pH on the glucose uptake by* H. diminuta *at 37° C during short interval incubations.*

pH	10^{-3}M uptake rate	Mean	pH	5×10^{-3}M uptake rate	Mean
6.8	109.5		6.9	123.7	
	79.0			109.1	
	79.1	76.3		174.7	124.2
	64.3			102.5	
	49.5			132.8	
				127.7	
7.1	59.5			99.0	
	66.7				
	80.5	72.7	7.1	244.3	
	93.5			165.2	193.6
	63.1			198.9	
				192.0	
7.5	64.5			167.4	
	73.0				
	72.4	71.4	7.4	161.2	
	64.8			165.0	164.3
	82.3			119.1	
				211.7	
7.9	107.4				
	47.5		7.6	156.3	
	58.9	77.2		104.7	
	94.9			99.7	167.4
				169.9	
8.3	51.1			145.5	
	63.3	73.5			
	106.1		7.9	209.4	
	73.6			197.7	
				182.7	
9.0	65.8			110.5	194.5
	46.0	60.1		230.9	
	77.2			242.4	
	51.3		8.4		
				253.3	
				246.1	
				178.7	
				168.6	212.6
				278.6	
				150.3	
			9.5	219.6	
				215.9	
				219.7	214.9
				204.7	

The uptake rates are expressed in terms of counts per minute per milligram of dry weight of worm tissue. Each rate represents that absorbed by an individual worm during a 60-second period.

The pH was varied in the same manner in a series of short interval experiments with 60-second incubations in labeled glucose (10^{-3} M). The results of these experiments (table III) indicate that in this time period there seems to be no effect of pH on the uptake rate. Experiments carried out with glucose at a concentration of 5×10^{-3} M gave similar results.

Since rate of diffusion should be directly proportional to the concentration of the solute, observations on concentration effects may indicate whether or not permeation is catalytic.

Therefore, a series of extended time experiments was carried out in which glucose uptake was measured with external concentrations of 1.11×10^{-3} M, 2.78×10^{-3} M, 8.34×10^{-3} M, 1.39×10^{-2} M, 2.78×10^{-2} M, 3.61×10^{-2} M, 4.44×10^{-2} M and

TABLE IV. *The effect of potassium ion concentration on the uptake of glucose by* H. diminuta *at pH 7.5 and 37° C.*

Number of worms	Glucose concentration (molarity)	K concentration (mM)	Mean uptake rate
3		0	80.3
3		5	93.8
3	5.6×10^{-2}	10	98.0
3		15	97.8
3		20	86.8
3		30	95.2
3		0	89.8
3		15	87.4
3	8.3×10^{-2}	30	85.5
3		45	87.9
3		60	85.7
3		0	75.7
3		15	83.0
3	1.12×10^{-2}	30	86.9
3		45	77.3
3		60	83.1

The uptake rates are expressed in terms of micrograms of glucose taken up per milligram of dry weight of worm tissue per hour.

5.55×10^{-2} M. As can be seen from figures 4 and 5, the glucose absorption of *H. diminuta* is independent of concentrations once outside the limiting concentration range. When the data are subjected to analysis by the method of Lineweaver and Burk (1934) in figure 6, the slope is found to be, approximately, 0.0000145. Thus, the apparent Michaelis constant is 1.65×10^{-3} M.

In short interval experiments, the concentration of glucose was varied from 5×10^{-5} M to 3×10^{-3} M. The worms were each incubated for 60 seconds at 37° C at pH 7.5. Figure 7 shows the effect of glucose concentration on the uptake rate. Using the Lineweaver and Burk treatment of the data (fig. 8), the apparent Michaelis constant was determined to be 1.53×10^{-3} M.

If chemicals, which are known to affect metabolism, decrease the rate of glucose absorption, the concept of a catalytic mechanism being involved in permeation would receive support. However, a qualification must be made; some substances tend to denature the constituents of living membranes and these constituents are not necessarily catalysts.

In extended time experiments, para-chloro-mercuribenzoate (PCMB) at a concentration of 4.4×10^{-3} M reduced the uptake rate from 111 micrograms of glucose per mg of dry weight per hour to 51 micrograms per mg per hour. The effect of dinitrophenol (DNP) was somewhat more pronounced. The control value for uptake was 103 micrograms per mg per hour; 1×10^{-5} M DNP reduced this to 77.1, 1×10^{-4} M DNP reduced it to 65.5 and 5×10^{-3} M reduced the value to 32.1.

Phlorizin has been reported by many authors to inhibit the absorption of glucose from the mammalian intestine, resorption in the kidney, and the passage of glucose into the mammalian erythrocyte. Laurie (1957) reported that phlorizin inhibits glucose fermentation by *Hymenolepis diminuta* in a non-competitive manner. A series of experiments was carried out in which the glucose concentration was varied. To vessels at each glucose concentration phlorizin was added to a concentration of 2.1×10^{-4} M, or 6.3×10^{-4} M. The results of this experiment are shown in figure 9. Since the ordinate intercepts are not common, the type of inhibition doesn't appear to be competitive; nor, since the slopes are not the same, does the inhibition appear to be non-competitive. This will be discussed in more detail below.

In short interval experiments with C^{14} labeled glucose, phlorizin was added at concentrations of 2.1×10^{-4} M, or 6.3×10^{-4} M. It can be seen from figure 10 that

the results of this short interval analysis corroborate those obtained in the extended time experiments.

Riklis and Quastel (1958) recently demonstrated a marked effect of potassium ion concentration on the glucose absorption rate of isolated loops of guinea pig intestine. Since they achieved such unequivocal results, it seemed desirable to test the effect of potassium ion concentration on the glucose uptake of *H. diminuta*. Three concentrations of glucose were used and at each concentration 5 concentrations of potassium ion were tested and compared. It can be seen from table IV that potassium concentrations in the range tested had no effect on the rates. In this particular series of experiments potassium salts were deleted from the control medium.

TABLE V. *The uptake of glucose by* H. diminuta *during its development.*

Number of worms		Worm age in days	Mean uptake rate	
Experiment A	Experiment B		Experiment A	Experiment B
5	15	8	83.2	115.9
15	15	12	146.9	171.5
15	15	16	118.2	118.1
15	15	20	85.2	113.7
15	15	90	89.2	102.2

These experiments were carried out at 37° C. and at pH 7.5, and the rates are expressed in terms of micrograms of glucose taken up per milligram of dry weight of worm tissue per hour.

Studies were made measuring the glucose uptake rates of tapeworms at various stages in their development to maturity in the definitive host. Worms were used 8 days, 12 days, 16 days and 20 days after infection of the rat host. Table V shows the results of 2 such experiments. Some data from 90-day samples are included to show that the values after 90 days are the same as those after 20 days.

In order to determine whether the effect noticed in the development experiment was due to an actual metabolic difference in the worms at that stage or whether, as is true in relation to mammals, size alone might have an effect upon the metabolic rate, size as a factor had to be studied. In an experiment originally designed to determine the effect of crowding on uptake of glucose, it was discovered that the rate of uptake of glucose by the worms is directly proportional to the weight of tissue over a very wide range. The data from this experiment are presented in table VI.

TABLE VI. *The effect of size of worm on the uptake of glucose by* Hymenolepis diminuta *at pH 7.5 and at 37° C.*

Number of worms per rat	Number of worms in each experiment	Mean weight of worms	Mean uptake rates and S.D.
5	14	102.6 mg.	96.7 (8.9)
10	15	75.9	116.6 (16.1)
20	14	38.0	122.1 (26.0)
40	15	27.2	86.2 (1.67)

The uptake rates are expressed in terms of micrograms of glucose taken up per milligram of dry weight of worm tissue per hour.

DISCUSSION

Temperature studies will not clearly demonstrate whether permeation of a substance is a catalytic phenomenon. Certainly studies utilizing only two temperatures are of dubious value. A high Q_{10} value might only be indicative of a slowly penetrating molecule. The relatively high amount of energy necessary for placing

the molecules in a reactive state might be indicative of a need for the participation of metabolic energy. It must be pointed out that in all this work the observed phenomena are tested in terms of the kinetics of purified systems.

After Wilbrandt and Laszt (1933) proved that Magee and Reid's (1931) increase in intestinal glucose absorption after addition of 0.2 percent sodium phosphate at pH 7 was due to pH and not to phosphate, Laszt (1935) tested the influence of other buffer solutions with hydrogen ion concentrations ranging from 10^{-4} to 10^{-8}. Only the solutions of pH 7 produced a marked increase in glucose absorption rate. Ponz and Larralde (1952) found that the rate of absorption of glucose by isolated loops of intestine was greatest at pH 7.5. These results and the data presented in this paper seem to suggest a mechanism other than simple diffusion since these slight variations in pH would hardly be severe enough to damage the membrane and thus account for the differences in uptake rates. The small secondary peak at pH 8.5 in the case of *H. diminuta* suggests the possibility of the action of more than 1 system.

The fact that changes in hydrogen ion concentration seemed to be without effect on the uptake of glucose in 60-second incubations is not readily explained. Since the substrate, glucose, is a non-polar compound, i.e., non-ionizable, results similar to those obtained by Read et al (1959) in their study of the uptake of amino acids might be expected. In the case of glucose permeation, perhaps the passage of the substrate is indirectly dependent upon processes within the worm which are very pH dependent. If this were the case, the effect of pH changes might not be seen in the initial 60-second period; i.e., the time required to change the internal pH would be longer than 60 seconds.

If permeation of glucose involved simple diffusion, the rates should rise relatively regularly with the increasing concentration. The results reported in this paper support the concept that glucose does not enter this tapeworm by simple diffusion, since the rate of absorption is independent of concentration above a certain level and is constant.

The fact that the metabolic inhibitors which were tested reduced the rate of glucose absorption by *H. diminuta* also suggests that the permeation process is related to the energy metabolism of the worm.

DNP, an uncoupler of oxidative phosphorylation, influenced glucose absorption in a relatively linear manner in terms of inhibition, suggesting that an energy mechanism may be the site of action of DNP on absorption.

At a concentration of 4.4×10^{-3} M, PCMB, a mercurial compound reacting with sulfhydryl groups, reduced glucose absorption by *H. diminuta* to 50 percent that of the control value. Bowyer and Widdas (1958) suggest that PCMB acts by causing denaturation of the membrane, although there has been no evidence to support this suggestion.

Experiments have not been carried out demonstrating reversal of the inhibition of glucose uptake in *H. diminuta*, but it seems relatively safe to suggest that the reduction in glucose absorption noted here is due to an inhibition of some catalytic process and not to a denaturation of natural proteins of the membrane.

Many workers have attempted to relate glucose absorption and phosphorylation by explaining that the inhibitory action of phlorizin on glucose absorption is a result of the effect of this inhibitor on phosphorylation and dephosphorylation

(Lundsgaard, 1933, and Ponz and Larralde, 1952). However, Jervis et al (1956) have recently shown that phlorizin inhibits glucose absorption at much lower concentrations than are necessary to inhibit alkaline phosphatase. In unpublished experiments, the present author has found the same to be the case in *Hymenolepis diminuta*.

The fact that phlorizin inhibits glucose absorption by the tapeworm in 60 seconds and the observation of Laurie (1957) that brief washing of the worms in Krebs-Ringer solution causes a reversal of inhibition of glucose fermentation, indicates that this glucoside acts at the surface of the worm. On the other hand, Read et al (1959) found that preincubation with certain other metabolic inhibitors was necessary to produce any inhibition of amino acid permeation in *Calliobothrium* during 2-minute incubations.

By extending the curves in figure 9 it was possible to obtain the intercepts of the 1/S axis. The intercept for the control was $-1/0.00088$, while that for 6.3×10^{-4} M phlorizin was $1/0.0079$, and that for 2.1×10^{-4} M phlorizin was $1/0.0069$. Since these values, also expressed as $-1/\mathrm{Km}$, would be identical if the inhibition were of the noncompetitive type, it was necessary to postulate that the inhibition might be competitive although the intercepts on the 1/V axis were not the same (this intercept or 1/Vmax should be common for both control and inhibitor curves). If the inhibition were competitive, the slope of the curve should equal $\mathrm{Km}/\mathrm{V}(1 + (\mathrm{I})/\mathrm{Ki})$ where V is Vmax. Ki was calculated from the equation which states that the intercept on the 1/S axis $= \dfrac{1}{\mathrm{Km}(1 + (\mathrm{I})/\mathrm{Ki})}$; the calculated value for Ki is 0.00437. By substituting in the equation for the slope, a value of 2.55×10^{-3} is obtained for the 6.3×10^{-4} M phlorizin curve. The observed value, i.e., that taken from figure 9, is 10^{-4}. The calculated slope for the 2.3×10^{-4} M phlorizin curve is 5.9×10^{-4}, whereas the actual slope taken from figure 9 is 5.1×10^{-5}. It can then be concluded that the inhibition of glucose absorption by the tapeworm *Hymenolepis diminuta* is neither competitive nor non-competitive in the definitive sense of these terms.

Riklis and Quastel (1958) demonstrated clearly that an increase in the concentration of potassium ions in the solution bathing the guinea pig intestine leads to an increase of the rate of glucose absorption. In *H. diminuta*, the external potassium ion concentration is apparently not important in the transport of glucose since, at each concentration of glucose tested, the uptake rate in the absence of potassium ions was not significantly different from that observed at any of the potassium concentrations used.

The fact that glucose uptake seems to be accelerated on the 12th day after infection, the time in the worm's development when differentiation is probably occurring at a maximum rate (Read and Rothman, 1957b), would be difficult to explain if the mechanism for the transport of glucose were a simple diffusion process and not a catalytic one. The data in table VI indicate that the effect seen on the 12th day after infection is not due to a size difference at that period in the development of the worm. Also, the latter findings validate the use of worms which are not the same size in any 1 experiment and in separate experiments where comparisons of rates are made.

Experiments being conducted at the present time indicate that the addition of an energy source prior to incubation in labeled substrate significantly increases the

uptake rates of worms which have been recovered from starved hosts, i.e. from hosts with a very limited supply of energy sources. Although more corroborative evidence must be compiled, one can conclude from the data presented in this paper that the movement of glucose into the tapeworm *H. diminuta* cannot be explained in terms of simple diffusion, but must be accounted for in terms of a catalytic process which seems to be dependent upon the energy metabolism of the tapeworm.

SUMMARY

A study of some of the factors influencing glucose absorption by *Hymenolepis diminuta* indicates that the process of permeation cannot be accounted for by simple diffusion, but is more complicated and probably involves metabolic processes.

The Q_{10} values and the amount of energy necessary for placing glucose molecules in a reactive state suggest that metabolic energy might be involved in some manner in the permeation process.

Although pH does not affect the absorption of glucose by this worm in short interval experiments, in extended time experiments an optimum of absorption occurs at pH 7.5.

The concentration of glucose above 1.1×10^{-2} M has no effect upon the rate of uptake and the apparent Michaelis-Menten constant is 1.5×10^{-3}M.

DNP in concentrations of from 10^{-5} M to 5×10^{-3} M reduces the rate of glucose absorption as does PCMB at a concentration of 4.4×10^{-3} M. Phlorizin in concentrations of 2.1×10^{-4} M and 6.3×10^{-4} M inhibits glucose absorption by *H. diminuta*. Lineweaver-Burk analysis yields results which can be interpreted as neither competitive nor noncompetitive inhibition. This inhibition by phlorizin acts at the surface of the worm.

The rate of glucose absorption by *H. diminuta* is significantly increased 12 days after infection over rates at 8, 16, 20 and 90 days after infection. The size of the tapeworm has no effect upon the rate of permeation of glucose across its membranes.

LITERATURE CITED

BOWYER, F. AND WIDDAS, W. F. 1958 The action of inhibitors on the facilitated hexose transfer system in erythrocytes. J. Physiol. **141:** 219–232.

JERVIS, E. L., JOHNSON, F., SHEFF, M. F. AND SMYTH, D. H. 1956 The effect of Phlorhizin on intestinal absorption and intestinal phosphatase. J. Physiol. **134:** 675–689.

LASZT, L. 1935 Die resorption von glucose und xylose bei verschiedener H^+ konzentration. Biochem. Z. **276:** 40–43.

LAURIE, J. 1957 The in vitro fermentation of carbohydrates by two species of cestodes and one species of Acanthocephala. Exp. Parasit. **6:** 245–260.

LINEWEAVER, H. AND BURK, D. 1934 The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56:** 658–666.

LUNDSGAARD, E. 1933 Hemmung von Esterifizierungs vorgöngen als Ursache die Phlorrhizinwirkung. Biochem. Z. **264:** 209.

MAGEE, H. E. AND REID, E. 1931 The absorption of glucose from the alimentary canal. J. Physiol. **73:** 163.

NELSON, N. 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153:** 375–380.

PONZ, F. AND LARRALDE, J. 1952 Relation of selective absorption of sugars and some enzyme inhibitors. Rev. Espan. Fisiol. **8:** 71.

READ, C. P. 1955 Intestinal physiology and the host parasite relationship. Pp. 27–43 *in* Cole, W. H. Some Physiological Aspects and Consequences of Parasitism. Rutgers University Press, New Brunswick, N. J.

READ, C. P. AND ROTHMAN, A. H. 1957a The role of carbohydrates in the biology of cestodes.

II. The effect of starvation on glycogenesis and glucose consumption in *Hymenolepis*, Exp. Parasit. **6**: 280–287.

READ, C. P. AND ROTHMAN, A. H. 1957b The role of carbohydrates in the biology of cestodes. IV. Some effects of host dietary carbohydrate on growth and reproduction of *Hymenolepis*. Exp. Parasit. **6**: 294–304.

READ, C. P., SIMMONS, J. E. AND ROTHMAN, A. H. 1959 Permeation and membrane transport in animal parasites: Amino acid permeation into tapeworms from elasmobranchs. J. Parasit. In press.

RIKLIS, E. AND QUASTEL, J. H. 1958 Effects of cations on sugar absorption by isolated surviving guinea pig intestine. Can. J. Biochem. and Physiol. **36**: 347–362.

SIMMONS, J. E., READ, C. P. AND ROTHMAN, A. H. 1959 Permeation and membrane transport in animal parasites: Permeation of urea into cestodes from elasmobranchs. J. Parasit. In press.

WILBRANDT, N. AND LASZT, L. 1933 Untersuchen über die ursachen der selektiven resorption der zucker aus dem darm. Biochem. Z. **259**: 398–417.

EXPLANATION OF PLATE I

FIGURE 1. Effect of temperature on the rate of glucose uptake by *Hymenolepis diminuta* at pH 7.5 and 8.3×10^{-3} M. These experiments were carried out over a 30-minute period. K refers to rate of uptake of glucose in micrograms per mg of dry weight of worm per hr. T is the absolute temperature in degrees Kelvin.

FIGURE 2. Effect of temperature on the rate of absorption of glucose by *Hymenolepis diminuta* at pH 7.5 and 5×10^{-3} M. These experiments were carried out over a 60-second period. K refers to uptake of glucose in counts per minute per mg of dry weight of worm. T is absolute temperature in degrees Kelvin.

FIGURE 3. The effect of pH on the rate of glucose uptake by *Hymenolepis* at 37° C and 8.3×10^{-3} M. The time of incubation was 30 minutes. Each point is an average of 4 experiments.

FIGURE 4. The effect of glucose concentration on its uptake by *H. diminuta* at pH 7.5 in 30 minutes. Each point is the mean of 7 experiments except for 4.4×10^{-3} M.

FIGURE 5. The relation of initial concentration of glucose to the relative amount absorbed at each concentration. Each value refers to the mean of 7 experiments.

FIGURE 6. A double reciprocal plot of molarity of glucose (s) and rate of uptake (v) of glucose over a 30-minute period.

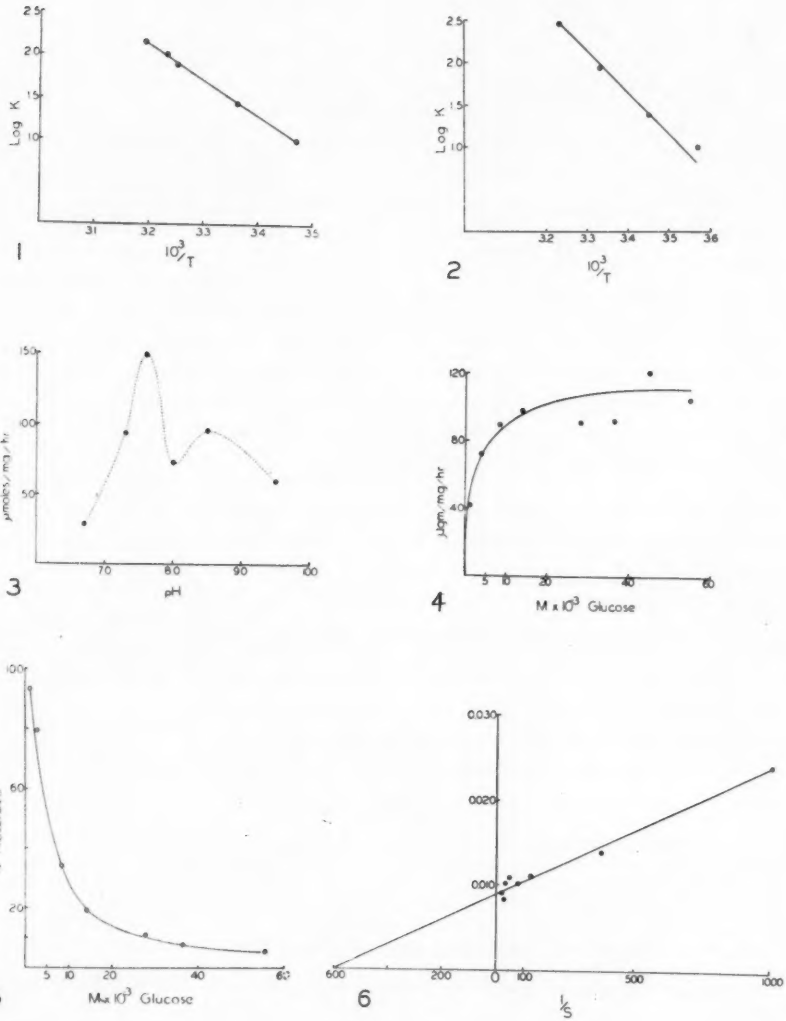

Log K
$10^3/T$
Log K
$10^3/T$
μmoles/mg./hr
pH
μgm./mg./hr
M x 10^3 Glucose
% Absorbed
M x 10^3 Glucose
1/S

PLATE I

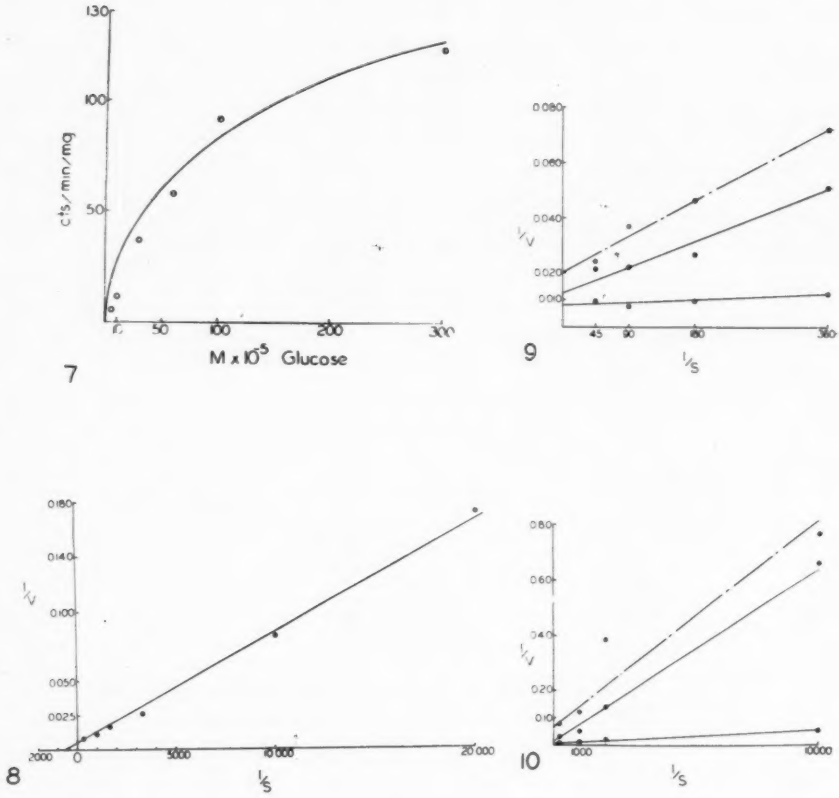


PLATE II

Explanation of Plate II

Figure 7. The effect of glucose concentration on its uptake by *Hymenolepis diminuta* in 60 seconds.

Figure 8. A double reciprocal plot of glucose concentration (s) and rate of its absorption (v) by *H. diminuta* for a 60-second period.

Figure 9. A Lineweaver-Burk plot showing the effect of phlorizin on the uptake of glucose by *H. diminuta* in 30 minutes. The lower line represents the control, the middle line shows the effect of 2.1×10^{-4} M phlorizin and the upper line shows the effect of 6.3×10^{-4} M phlorizin. V refers to the uptake of glucose in micrograms per mg of dry weight of worm per hour. S is molarity of glucose.

Figure 10. A Lineweaver-Burk plot showing the effect of phlorizin on the uptake of C 14-glucose by *H. diminuta*.

EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE EGGS OF *ASCARIDIA GALLI**

W. MALCOLM REID

Poultry Department and Poultry Disease Research Center, University of Georgia, Athens, Georgia

The intestinal roundworm, *Ascaridia galli* (Schrank, 1788), continues to present an economic problem to broiler growers in southeastern United States in spite of the ever decreasing growing period required to produce a 3-pound broiler. In recent studies (Reid, 1958) samples of 10 birds from each of 100 farm flocks showed 59 percent of the flocks and 27.5 percent of the birds carried *A. galli* at marketing time when birds averaged about 9 weeks of age. Large expenditures for worm treatment have proved disappointing in the amount of control thus achieved. Earlier studies (Ackert, 1931) suggested that the life cycle of *A. galli* required up to 50 days, but many birds are now marketed between 56 and 70 days, thus making it difficult for a life cycle of that duration to be completed. A restudy of the life history of the parasite thus seemed desirable to answer the riddle of the survival of the parasite within these short growing periods and to give basic information useful in planning control programs.

Kerr (1955), in a series of experiments which were devised to establish the minimum maturing time of the worm in birds of different ages, showed that fertile eggs were recovered by fecal examinations as early as 30 days after infective eggs were ingested by 12-day-old chicks. Somewhat longer periods were required for older birds. Roberts (1937) found fertile eggs in the intestine as early as 27 or 28 days after infection with a 100 *A. galli* eggs. The 50-day period reported by Ackert would appear to be the average maturing time in somewhat older birds. No similar study on the minimum maturing time for eggs to develop to infectivity has been reported in the literature. Such work, together with temperature studies as related to development in the litter of brooder houses, is here reported.

MATERIALS AND METHODS

Fertile eggs were maintained in Petri dishes using water suspensions according to methods reviewed by Reid, Pate and Kleckner (1958). Petri dishes were placed in small incubators within 2 hours of removal of the female worms from the host. A series of experiments was designed in which thermostats were regulated to within ±2° F for the following temperatures: 99°, 97°, 95°, 93°, 90°, 86°, 82°, 79°, 75°, 70°, 68°, 66°, and 64° F. For colder temperatures, it was necessary to maintain the incubator in a cooled room. Eggs were aerated daily by rotation of the dishes after removal of the cover. An attempt was made to parasitize a group of 5 2-week old chicks on each of a series of successive days as soon as larvae appeared infective on microscopic examination. Minimal infective time was determined by repeating the work in a daily series of inoculations until a day was found on which birds became infected following a day when no birds were infected. For brevity in reporting these results, only the earliest day of infectivity is recorded. This indicates that 2 or more of the 5 birds contained worms after incubation for this period and for longer periods. It also indicates that 5 birds were negative for *A. galli* when eggs were removed 1 day earlier than the reported period.

Received for publication April 21, 1959.

* Journal Series Paper No. 102, College Experiment Station, University of Georgia, Athens, Georgia. This study was partially supported by a research grant from the Moorman Manufacturing Company of Quincy, Illinois.

Litter temperature recordings were made simultaneously from two locations in a typical brooder house for 3 different broods of birds at different seasons of the year. The 1st group was housed for 64 days in the spring between April 17 and June 19; the 2nd group in the summer for 70 days between July 11 and September 19; and the 3rd group, which were replacement pullets, for 125 days from November 30 to April 16. A Minneapolis-Honeywell, 2-pen temperature recorder was used with two 5-foot, mercury-filled thermal systems equipped with stainless steel bulbs. Recording charts were changed at weekly intervals. One bulb, attached to pen No. 1, was placed under the brooder hover 6 to 10 inches from the edge while the other (pen No. 2) was placed 1 foot from the outside wall of the brooder house. Temperature records were kept either at the surface or as deep as 4 inches under the pine-shavings litter. Brooder houses were of the lightest construction used in the North Georgia area. Side walls consisted of but a single layer of tar paper in the winter and wire mesh only in the summer.

RESULTS AND DISCUSSION

Minimal Maturing Time

Normal development from the one-celled stage to fully infective larvae did not occur at 99° F in 3 trials, at 97° F in 1 trial, or at 95° F in 3 trials. These results are similar to reports of Roberts (1937) who found no larvae matured at 95° to 99.5° F. Early cleavage forms (Ackert, 1931) could often be found, but normal development soon ceased. Development occurred rapidly at 93° F and was completed in 5 days as demonstrated by infection experiments. It may be concluded that 93° F is the maximum temperature which permits development and that temperatures above this level are lethal. Embryonic development was also completed in 5 days at temperatures of 90° F as indicated by other infection experiments.

As the temperature was lowered, developmental time increased as follows: 86° F, 6 days; 82° F, 8 days; 79° F, 9 days; 75° F, 13 days; 70° F, 19 days; and 68° F, 19 days. The lowest temperature at which developmental stages could be observed was 66° F. Infection did not occur when embryonated eggs maintained at this temperature were fed in 3 different experiments between 20 and 32 days. In a fourth experiment, eggs were not infective at 20 days, but infective after 32 days of incubation at 66° F. The minimum maturing time was not determined in this experiment. It was thus shown that 66° F is the minimum temperature at which development could occur. Below this temperature, larval development was arrested completely. Eggs at 64° F remained viable and later developed to infectivity after being warmed up. Other investigators (Ackert, 1931; Ackert and Cauthen, 1931; and Roberts, 1937) have studied the effects of colder temperatures on embryonated eggs. Their ability to survive freezing temperatures and to complete development after warming up depends upon the length of exposure to cold and the degree of development at the time of exposure.

The minimum time of 5 days indicates a more rapid development than has previously been reported. Roberts (1937) records development in as short a time as 7 days, while Ackert (1931) indicated a minimum of 10 days. This 5-day minimum, together with the 30-day developmental time in the bird (Kerr, 1955), indicates that the entire life cycle may be completed in 35 days. Thus, it is possible that a second generation of worm eggs may develop, at least as far as the tissue penetration stage, before marketing time. The most harmful effects of this parasite occur during this life cycle phase between 10 and 17 days after ingestion of embryonated eggs.

Litter Temperature

A study was initiated to determine actual temperatures under practical broiler

house conditions. Two continuous recordings of the temperature in the litter were made for a total of 239 days during development of spring, summer and winter broods of chickens. Table I gives comparisons between these means and ones obtained from the U. S. Weather Bureau which was located about 2 miles from the brooder house. Recordings for each brood were divided into 2 periods. The 1st period included mean temperatures recorded during the time when artificial heat was provided under brooder hovers in the house. This period was 29 days in the spring, 21 days in the summer, and 37 days in the winter broods. No other supplemental heat was used in the house at any time. The second period included recordings when there was no artificial heat in the house and the brooder was pulled to the ceiling for storage by a system of ropes and pulleys. All recordings showed higher temperatures in the litter than that indicated outside by Weather Bureau recordings. The least increase (0.4° F) over outside temperatures was recorded

TABLE I. *Temperature means in brooder house litter compared with the U.S. Weather Bureau means for the same periods. Temperatures in degrees F.*

	Recording pen No. 1 under hover litter	Increase over Weather Bureau mean	Recording pen No. 2 brooder-house litter	Increase over Weather Bureau mean
Gas brooder and hover in operation 29 days beginning April 17, 1956	107.1*	41.5	70.3	4.7
No artificial heat for 35 days beginning May 16	84.1	10.7	76.5	3.1
Gas brooder and hover in operation 21 days beginning July 11, 1956	79.8	0.4**	82.8	8.4
No artificial heat for 49 days beginning August 1	80.1	2.2	77.1	0.8
Gas brooder and hover in operation 37 days beginning November 30, 1956	70.4	19.6	57.1	5.3
No artificial heat for 88 days beginning January 1	73.5	21.2	61.8	9.5

* An improperly regulated brooder sent temperatures as high as 140° F for a short time.
** Thermometer bulb buried 4 inches in the litter.

in the summer months under the hover when the thermometer bulb was buried under 4 inches of litter. The most noteworthy increase over outside temperatures was recorded in the under-hover area during the period while there was no artificial heat in the house and brooders were hoisted to the ceiling. This increase, which was at first puzzling to the investigator, was greatest in winter with an average increase over outside conditions of 21.2° F. During the week starting February 15, it averaged 42.1° F higher than Weather Bureau recordings. A study of a sample recording for the week starting February 26, which was 7 weeks after use of artificial heat had been discontinued (fig. 1), makes the reason apparent. It will be noted that the temperature went up to about 100° F every night but dropped to the level of the other litter areas in the day-time. Observation of the birds showed that they habitually huddled in this area in the center of the house and thus warmed up the litter themselves. This hovering habit also showed up in the recordings during the spring, but not during the summer brood. This latter group of birds never learned to use the brooder during warm summer nights. However, there were other areas within the house where they habitually rested both by day and by night. It may be assumed that similar rises in litter temperature occurred in these areas. The crowding which often results in less than 1 sq ft of floor space

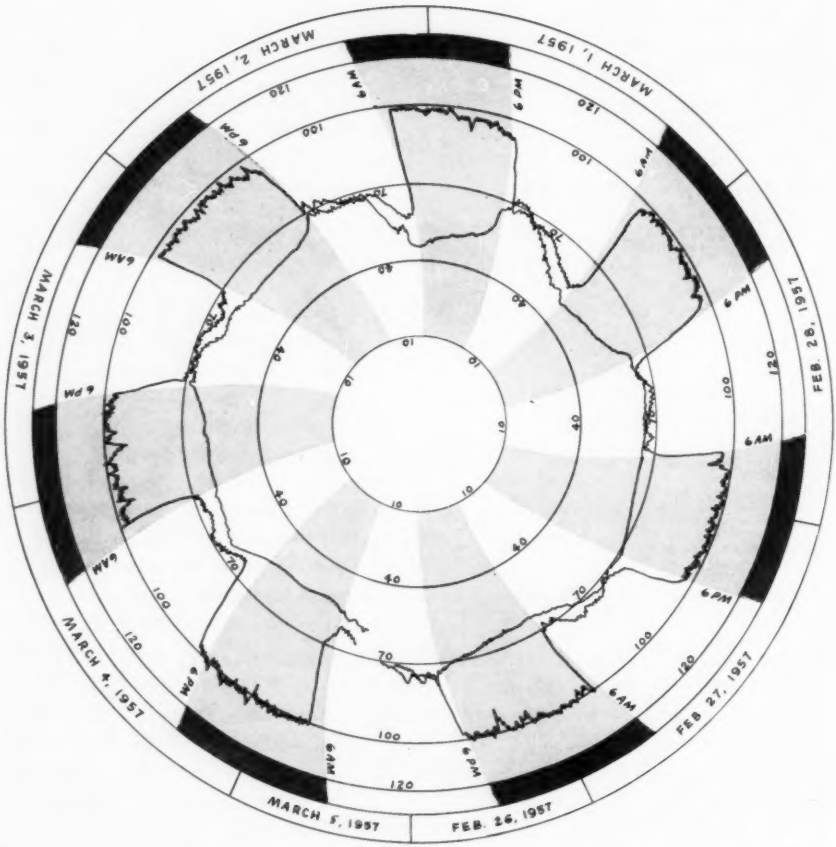


Fig. 1. Litter temperature fluctuations in a brooder house 12½ weeks after birds were started and 7 weeks after artificial heat was discontinued. Inner tracing (Pen #2) recorded temperature in the litter one foot from the outside wall of the house. The outer tracing (Pen #1) recorded marked increases in temperature at night (shaded areas) as the birds huddled by habit in the former brooder-hover area.

per bird means that large areas of these densely populated brooder houses may have litter warmed up by resting birds.

Comparison of temperature recordings from different litter depths indicates that litter is a fairly good insulating medium. In some cases where surface temperatures under the hover registered 95° F, the recorded temperature in the same area but 4 inches under the litter averaged 5 to 15 degrees lower. Thus, the litter acts as a heat distributing agent, and many different temperatures will be present in a given house at a given time. Body temperature of the bird is some 11° F too warm for eggs to develop, as determined in the present studies, but there are adjacent areas where temperatures are close to 93° F, the optimum for rapid egg development. The body heat of the bird thus hastens the developmental cycle of the parasite under brooder house conditions. The birds are literally assisting in the incubation of the nematode eggs by warming them with their own body heat. In some areas of the house, temperature conditions approach the optimum developmental temperature. This heating of litter by the birds themselves is no doubt an important ecological factor in the high incidence of this parasite under brooder-house conditions.

SUMMARY

1. Minimum time required for *Ascaridia galli* eggs to reach infective stage after being passed from the bird is 5 days at 93° F (34° C) and 90° F (32° C). Above 95° F (35° C) normal development ceases. Development continues at a slower rate as temperature is reduced with the minimum temperature being 66° F (19° C). Below this, development is arrested, but may continue upon warming. The five day minimum maturing time to the infective stage is at least 2 days earlier than indicated by other investigators.

2. Near optimum litter temperatures for larval development prevail in poultry houses where confinement rearing is practiced. This is due to body heat from the birds when hovering or resting on the litter, which produces sufficient heat to induce rapid larval development within the egg.

LITERATURE CITED

ACKERT, J. E. 1931 The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). Parasit. **23:** 360–379.

ACKERT, J. E. AND CAUTHEN, G. E. 1931 Viability of the eggs of the fowl nematode, *Ascaridia lineata* (Schneider). J. Parasit. **18:** 113.

KERR, K. B. 1955 Age of chickens and the rate of maturation of *Ascaridia galli*. J. Parasit. **41:** 233–235.

REID, W. M. 1958 Incidence of helminth and external parasites in Georgia broilers. Poul. Sci. **37:** 386–392.

REID, W. M., PATE, D. D. AND KLECKNER, A. L. 1958 Effects of *Ascaridia galli* on chicks with infectious bronchitis. Avian Dis. **2:** 99–109.

ROBERTS, F. H. S. 1937 Studies on the biology and control of the large roundworms of fowls, *Ascaridia galli* (Schrank, 1788) Freeborn 1923. Queensland Agr. J. **47:** 8–16.

RESEARCH NOTE

AN UNUSUAL CASE OF HUMAN POROCEPHALIASIS.

On May 12, 1959, the senior author was called into consultation at Duke Hospital for identification of a parasite floating free in the anterior chamber of a patient's eye. Examination in situ, verified by study of the specimen after removal from the eye, revealed an unencapsulated third-stage larval pentastomid, *Linguatula serrata* Frölich (See figure 1).

The patient was a sixteen-year-old negro boy from Danville, Virginia. He first noticed the pentastomid about 1 month prior to his admission to Duke Hospital. The presence of the parasite in the anterior chamber of his eye apparently caused no pain, the white spot being evident when he looked carefully in a mirror to see why his eye was a "little red."

Detailed questioning of the patient revealed no clues as to the source of his parasite. City water was used in his home. The boy denied close association with dogs or other animals on the home place. He has never gone swimming, but does enjoy fishing. We do not know about the food habits of his family. The source of the egg or early larva from which this third-stage larva developed remains a moot question.

There was no other evidence of parasitism in the patient, although thorough examinations of the stools, respiratory tract, and sinuses were made when the patient was hospitalized.

We are grateful to Dr. Banks Anderson of Duke Hospital for calling this unusual case to our attention.—WANDA SANBORN HUNTER AND ROBERT P. HIGGINS, *Zoology Department, Duke University, Durham, North Carolina.*

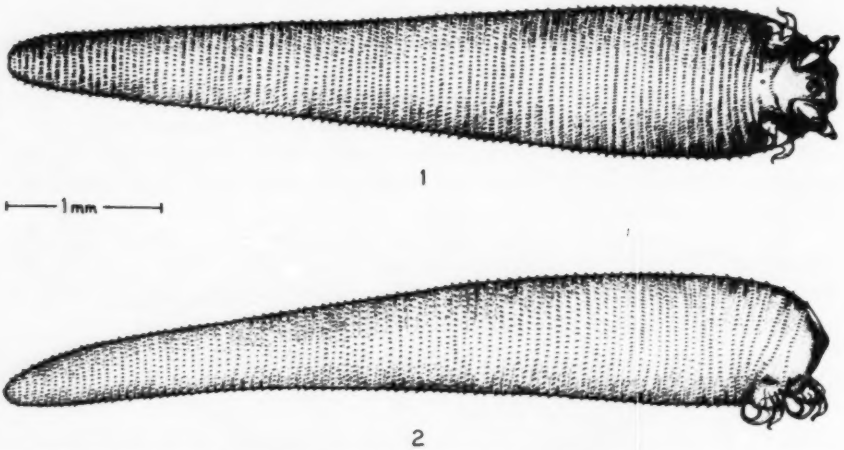


FIGURE 1. *Linguatula serrata* removed from anterior chamber of a human eye. 1, ventral view; 2, lateral view.

THE FINE STRUCTURE OF THE GROWTH REGION OF OVARY IN *ASCARIS LUMBRICOIDES* VAR. *SUUM* WITH SPECIAL REFERENCE TO THE RACHIS*

JAMES J. PRESTAGE**
Department of Zoology, State University of Iowa, Iowa City, Iowa

Studies on the structure of the reproductive system in female *Ascaris* probably had their beginning with Eschricht (1848). Since then, the system has been studied by numerous investigators among whom were von Kemnitz (1912), Fauré-Fremiet (1913), Musso (1930), Chitwood and Chitwood (1940), and Ishii and Yanagisawa (1954). These studies have contributed greatly to our knowledge of the structure of the reproductive sytem and oogenesis in *Ascaris;* however, not all investigators are agreed as to the origin and significance of the rachis which is first seen arranged as a branched body in the upper growth region of the gonad and later as a circular body in the middle and lower regions of the gonad.

It is the object of this study to reinvestigate the growth region of the ovary with the electron microscope in the hope of obtaining further information pertaining to the origin and significance of the rachis and the fine structure of the ovary in this region.

MATERIALS AND METHODS

The worms, *Ascaris lumbricoides* var. *suum,* used in this study were provided by the Wilson Packing Company of Cedar Rapids, Iowa. The larger worms, 20 cm and longer, were used for electron microscopy. This was necessary because of the difficulty encountered in locating the finest portions of the system.

Body fluid, to be used as a dissecting medium, was collected from a number of worms. This was done by cutting off the posterior tip of the worms and very gently milking them in an anterior-posterior direction to force out the body fluid. The reproductive system was removed from the worm and placed in the body fluid medium where the desired portions of the system were removed. The selected portions were fixed for 2 hours in 1 percent osmium tetroxide solution buffered to a pH of 7.3 to 7.5 (Farquhar, 1956), dehydrated in graded concentrations of alcohol, infiltrated and embedded in methacrylate (Newman, Borysko and Swerdlow, 1949, and Farquhar, 1956). After polymerization, the tissues were sectioned with a Porter-Blum microtome set to cut at 0.025 microns, placed on specimen grids coated on one side with a thin Formvar film, and examined with the RCA EMU 3D electron microscope.

OBSERVATIONS

Figure 1 is a longitudinal section through the lower region of the germinal zone. The germ cells of this region contain a few lipid droplets (LD). The presence of lipid droplets may serve to indicate that the cells are no longer dividing and have begun to store nutrients.

Electron micrographs of sections through the upper region of the growth zone

Received for publication April 23, 1959.

* Drawn from a dissertation in Zoology presented to the Faculty of the Graduate School of the State University of Iowa in partial fulfillment of the requirements for the degree Doctor of Philosophy. This study was made under the supervision of Dr. H. W. Beams. Thanks are due to Dr. L. O. Nolf also.

** Recipient of a Fellowship from the National Medical Fellowships, Inc., through funds appropriated by the National Foundation. Present address: Biology Department, Southern University, Baton Rouge, Louisiana.

reveal that the primary oocytes are in protoplasmic continuity with the branched axes of the rachis (figs. 2, 3 and 4, R). All of the primary oocytes in the electron micrographs are not shown to be in protoplasmic continuity with the rachis, but such a relationship is suggested by their being oriented toward the rachis. Where this continuity is not evident, the rachis appears as a separate entity having a limiting membrane (RM) that is approximately 20 millimicrons thick. The membrane is quite evident in those places where a cell membrane (CM) lies adjacent to that of the rachis. The widths of the axes (figs. 2 and 3) are approximately 1.7 microns and 2 microns, respectively. The rachis contains mitochondria (M), endoplasmic reticulum (ER) and glycogen particulates (GP). Numerous oriented fibrous-like structures are seen at the point of junction of the primary oocytes with the rachis; under high magnification they appear to be oriented strands of endoplasmic reticulum (Fig. 4, ER).

Except for the presence of a nucleus, the primary oocyte contains the same cytoplasmic inclusions and organelles observed in the rachis. The nucleus of the primary oocyte displays a double membrane whose total thickness is approximately 20 millimicrons (fig. 4, N).

Figures 5 and 6 are electron micrographs of sections through the growth zone where the rachis has assumed a cylindrical shape. Here the limiting membrane of the rachis was measured and found to have a thickness of approximately 40 millimicrons. There is an increase in the quantity of nutritive inclusions in the rachis, with lipid droplets (LD) being the most obvious. The largest lipid droplet was approximately 1.2 microns in diameter. Small osmiophilic droplets (OD) and glycogen particulates (GP) are scattered throughout the rachis. Figure 6 shows a mitochondrion lying in part of both the rachis and a primary oocyte. Its direction of movement is not known. Mitochondria and endoplasmic reticulum are randomly distributed in the rachis.

The primary oocytes accumulate large quantities of nutritive inclusions which are concentrated mostly in the basal portion of the cells (fig. 7). The larger lipid droplets average around 2.25 microns in diameter, whereas the larger yolk platelets (YP), which appear to be composed of numerous glycogen particulates (GP), average around 2.5 microns in diameter (fig. 8). Small osmiophilic droplets, glycogen particulates, mitochondria and endoplasmic reticulum are randomly disposed in the cytoplasm of the cells. The nucleus of the cells is also situated in the basal portion. The nucleus (N) contains fragments of several nucleoli (figs. 7 and 9).

Epithelium. The epithelial cells (EC) in the lower region of the germinal zone and upper region of the growth zone contain numerous mitochondria which give them the appearance of being granular (fig. 1, 10 to 12, M). The epithelial cells in the upper region of the growth zone also contain secretory vesicles (figs. 11 and 12, SV). The vesicles have what appears to be a distinct membrane (VM) enclosing a substance which was shown by histochemical means to be glycogen. In each cell examined, it was observed that the secretory vesicle had displaced the nuclei (EN) towards the periphery of the cell. Scattered throughout the cytoplasm are glycogen granules (GG) and endoplasmic reticulum (ER) along with the large number of mitochondria (M). The cells are attached at their broad base to the ovarian envelope (OE).

Figures 13 to 15 are electron micrographs of epithelial cells observed in other regions of the growth zone. The cells are generally cylindrical in form, but occa-

sionally a drumstick type is seen (fig. 14). Electron micrographs of these cells revealed them to contain the same cytoplasmic organelles observed in other epithelial cells. The secretory vesicles are absent and the concentration of nutrient inclusions diminished. In cross-sections of these cells the nuclei are very irregular in shape. In a longitudinal section through the epithelial cells, the nuclei and the mitochondria are oriented with the longitudinal axis of the cell (fig. 16).

Between the epithelial cells and the ovarian envelope are structures of varying heights and widths (Figs. 13 to 17, P). The structures are reminiscent of the podocyte extensions observed in the renal corpuscles of vertebrates (Prestage and Beams, 1957). However, unlike the podocyte extensions, they do not appear to form a continuous layer on the inner margin of the ovarian envelope. The structures make periodic contact with the basal cell membrane, holding parts of it away from the ovarian envelope. These structures appear to consist of small fibers (fig. 17).

Ovarian Envelope. The ovarian envelope (OE) appears to be composed of an amorphous substance (AS) and fibers (F) (figs. 10 to 12). The ovarian envelope in the upper region of the growth zone contains scattered fibers, but as the ovary was observed more distally the number of fibers was progressively increased, giving the ovarian envelope a laminated appearance (figs. 13 to 17).

DISCUSSION

Rachis. Eschricht (1848) observed that the germ cells in the female *Ascaris* are not free in the gonoduct, but are grouped around a central axis termed the rachis. This phenomenon has also been observed in some oxyurids, strongylins and spirurids; but, as pointed out by Seurat (1920), the rachis is not a constant feature of nematodes, even in special groups. The apparently erratic occurrence of the rachis has created much interest in its origin and significance.

Chitwood and Chitwood (1940) stated that there are two possible origins of the rachis: one, as a continuation and product of the terminal cell; the other, as a residuum of enucleated plasma separated off from a germinal syncytium. A similar opinion was expressed by Fauré-Fremiet (1913) who believed that the rachis arose from an incomplete division of cellular bodies of the primitive germinative cells, mother cells and gonia, and would constitute some kind of a cytoplasmic reserve or a syncytial mass destined to disappear progressively. Ishii and Yanagisawa (1954) posed the possibility of the rachis being differentiated from the intercellular substance which fills the spaces between the oogonia.

Electron micrographs of longitudinal sections through the germinal zone of the ovary give no indication of the rachis being present. This observation tends to eliminate the possibility of rachis being a continuation of the terminal cell. It also suggests that the rachis does not arise from an incomplete division of cellular bodies, since the germ cells are initially separated from one another.

The writer is of the opinion that the rachis has its origin shortly after the first germ cells are formed. This opinion is based on the growth of the ovary of *Ascaris* which is comparable to that of a root in that cell division occurs in the vicinity of the blind end. These cells, which are derived from the terminal cell, undergo further division and are left behind as the terminal cell continues to divide extending the length of the ovary. Since electron micrographs reveal no other structure among the germ cells, it seems reasonable to assume that during the early stages of growth

of the germ cells a syncytium is formed giving rise to the rachis. The rachis is extended in the direction of the germinal zone as a result of the newly formed cells becoming a part of the syncytium.

The significance of the rachis is not understood. Bütschli (1873), Mayer (1908), and others expressed the belief that the rachis of *Ascaris* is comparable in a certain measure to Verson's cell of lepidopterans, the implication being that the rachis is a cell having a nutritive function. However, Chitwood and Chitwood (1940) asserted that the rachis, to which the oocytes are attached, is not nucleated and hence cannot subserve a nutritive function. Also of this opinion was Seurat (1920), who contended that the rachis does not supply yolk to the eggs.

The ground substances and inclusions of the rachis and primary oocytes, as shown by the electron microscope, are inseparable and appear as one continuous mass. On the basis of this observation, there appear to be two possible functions of the rachis: (1) serving as a reservoir into which certain constituents of the primary oocytes are expelled; (2) serving as a reservoir that is capable of absorbing and synthesizing nutrients which are used to facilitate the acquisition of nutrients by the primary oocytes. Since the primary oocytes are in quest of nutritive materials, the writer favors the latter as the probable function of the rachis.

SUMMARY

The rachis was observed as a branched body in the upper region of the growth zone and as a circular body in the middle and lower regions of the growth zone. Germ cells were observed to be in protoplasmic continuity with the rachis in each region examined. In the upper region of the growth zone, the rachis contains a few scattered mitochondria, endoplasmic reticulum, glycogen particulates, and lipid droplets. The quantity of organelles and inclusions was observed to increase as the rachis was examined in the middle and lower regions.

Evidence was obtained to eliminate the possibility of the rachis being a continuation of the terminal cell. Observations also suggest that the rachis does not arise from an incomplete division of cellular bodies. Hence, the rachis is thought to have its origin from germ cells forming a syncytium after being initially separated from one another.

The rachis is considered to be a reservoir whose function is to facilitate the intake of nutritive materials by the primary oocytes.

The epithelium of the ovary consists of a single layer of cells. The cells in the upper region of the growth zone were observed to contain secretory vesicles.

LITERATURE CITED

BÜTSCHLI, O. 1873 Beiträge zur Kenntnis der freilebenden Nematoden. Nova Acta der Kais. Leop. Carol. Deut. Akad. d. Naturf. Bd. **36**, No. 5.

CHITWOOD, B. G. AND CHITWOOD, M. B. 1940 An Introduction to Nematology. Baltimore, Maryland.

ESCHRICHT, D. F. 1848 Cited by Chitwood and Chitwood, 1940.

FARQUHAR, M. G. 1956 Preparation of ultrathin tissue sections for electron microscopy. Lab. Inv. **5**: 317–337.

FAURÉ-FREMIET, E. 1913 Le cycle germinatif chez l'*Ascaris megalocephala*. Arch. Anat. Micr. **15**: 435–758.

ISHII, K. AND YANAGISAWA, Y. 1954 Structure of the female reproductive organs of pig Ascaris. Jap. J. Med. Sci. Biol. **7**: 95–109.

VON KEMNITZ, G. 1912 Die Morphologie des Stoffwechsels bei *Ascaris lumbricoides*. Arch. Zellforsch. **7**: 463–603.

MAYER, A. 1908 Zur Kenntnis der Samenbildung bei *Ascaris megalocephala.* Zool. Jahrb. Abt. Anat. **25:** 495–546.

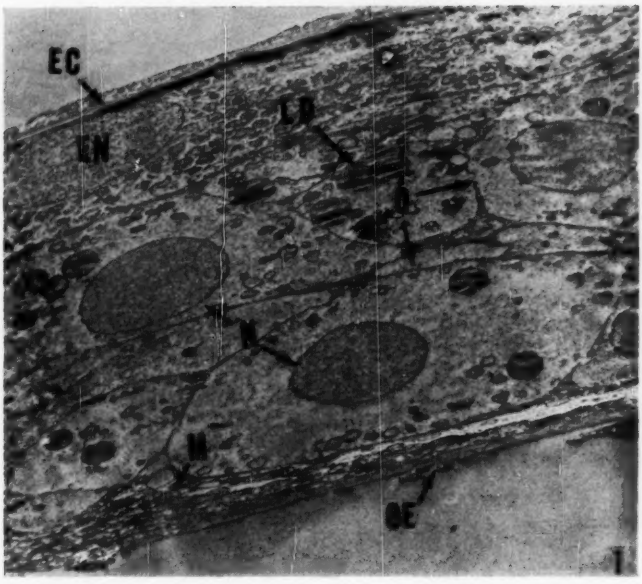
MUSSO, R. 1930 Die Genitalröhren von *Ascaris lumbricoides* und *megalocephala.* Ztschr. Wiss. Zool. **137:** 274–363.

NEWMAN, S. B., BORYSKO, E. AND SWERDLOW, M. 1949 New sectioning technique for light and electron microscopy. Science **110:** 66.

PRESTAGE, J. J. AND BEAMS, H. W. 1957 The structure of renal corpuscle in the sparrow, *Passer domesticus domesticus,* as revealed by the electron microscope. Iowa Acad. Sci. **64:** 670–679.

SEURAT, L. G. 1920 Histoire Naturelle des Nématodes de la Berbérie. Université d'Alger, Alger.

PLATE I



Explanation of Plates

Plate I

Figure 1. Longitudinal section through the lower region of the germinal zone showing oogonia (O) and epithelial cells (EC); oogonia containing lipid droplets (LD), and epithelial cells numerous mitochondria (M). ×4000.

Plate II

Figure 2. Cross section through upper region of the growth zone; showing an axis of the rachis (R) to which two primary oocytes are attached. Note rachis membrane (RM) and primary oocyte membrane (CM). ×5600.

Figure 3. Cross section through upper region of the growth zone; showing tip of an axis of the rachis (R) to which two primary oocytes are attached. ×5600.

Figure 4. Cross section through junction of a primary oocyte to rachis; rachis (R) containing endoplasmic reticulum (ER), glycogen particulates (GP) and mitochondria (M). Note orientated strands of endoplasmic reticulum (ER) at junction of oocyte with rachis and double membrane of nucleus (N). ×44,000.

Plate III

Figures 5 and 6. Cross sections through growth zone where rachis is a circular body; rachis containing glycogen particulates (GP), lipid droplets (LD), osmiophilic droplets (OD), mitochondria (M) and endoplasmic reticulum (ER). Note mitochondrion (M) lying in part of both a primary oocyte and the rachis in figure 6. ×14,500.

Plate IV

Figure 7. Longitudinal section through basal portion of a primary oocyte showing nucleus (N), mitochondria (M), lipid droplets (LD), osmiophilic droplets (OD), glycogen particulates (GP), and yolk platelets (YP). ×4800.

Figure 8. Section through oocyte showing lipid dropelts (LD), glycogen particulates (GP) and yolk platelet (YP). ×16,000.

Figure 9. Section through the nucleus (N) of primary oocyte containing fragments of several nucleoli. ×11,200.

Figure 10. Cross section through epithelial cells of the upper region of the growth zone showing large numbers of mitochondria (M). Note fibers (F) within amorphous substance (AS) of ovarian envelope (OE). ×5600.

Figures 11 and 12. Cross sections through epithelial cells of the upper region of the growth zone showing secretory vesicles (SV), glycogen granules (GG), endoplasmic reticulum (ER), mitochondria (M) and nuclei (EN). Note fibers (F) within amorphous substance of the ovarian envelope (OE). ×4,000.

Plate V

Figures 13, 14 and 15. Sections through epithelial cells of middle and lower regions of growth zone showing the various forms of the cells; cells containing irregular shaped nuclei (EN) and numerous mitochondria (M). Note the structures (P) between the epithelial cells and the laminated ovarian envelope (OE). ×5600.

Figure 16. Longitudinal section through an epithelial cell; nucleus (EN) and mitochondria (M) orientated with the longitudinal axis of the ovary. ×5600.

Figure 17. Cross section through the basal part of an epithelial cell and the ovarian envelope. Note epithelial cell membrane (CM) adjoining the structure (P) and the laminated ovarian envelope (OE). ×44,000.

PLATE II

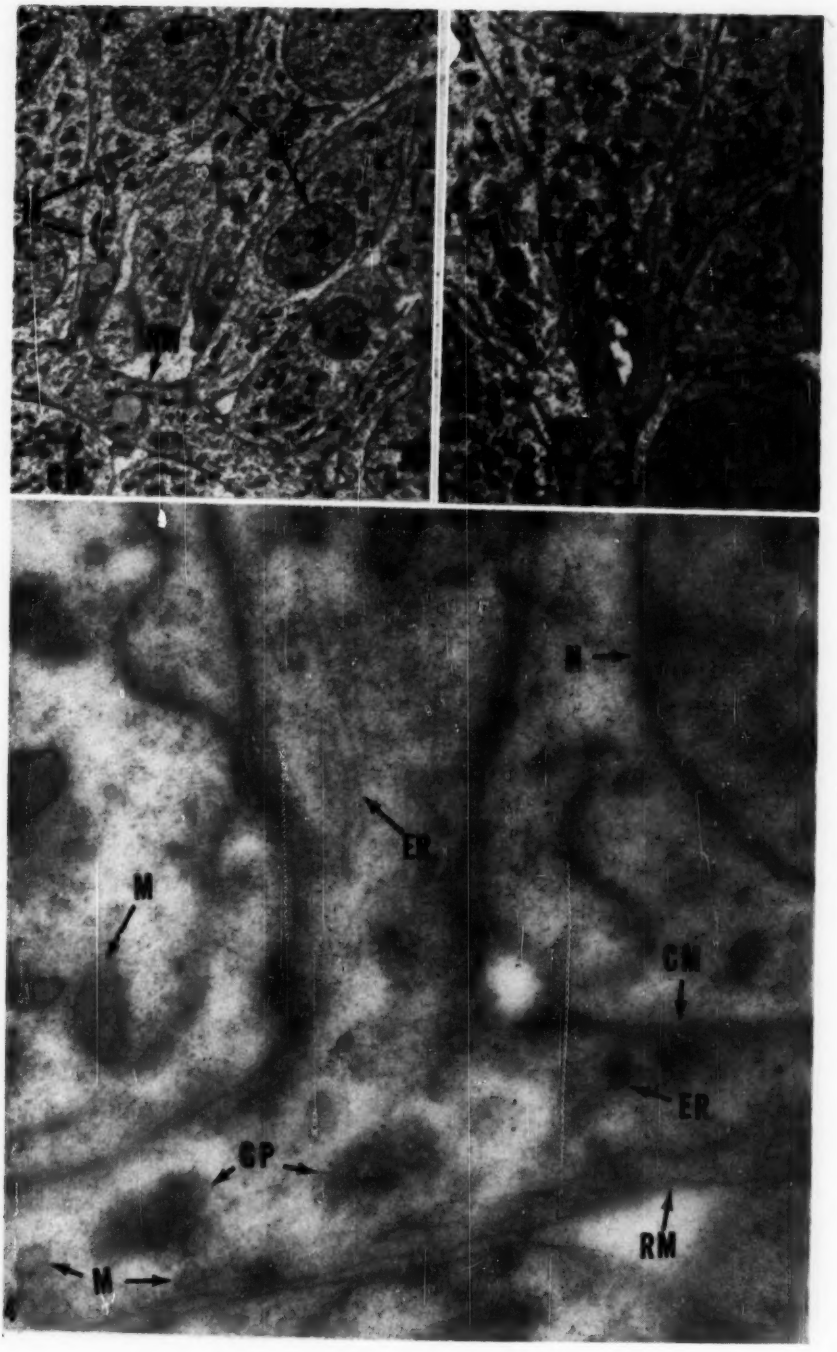


PLATE III

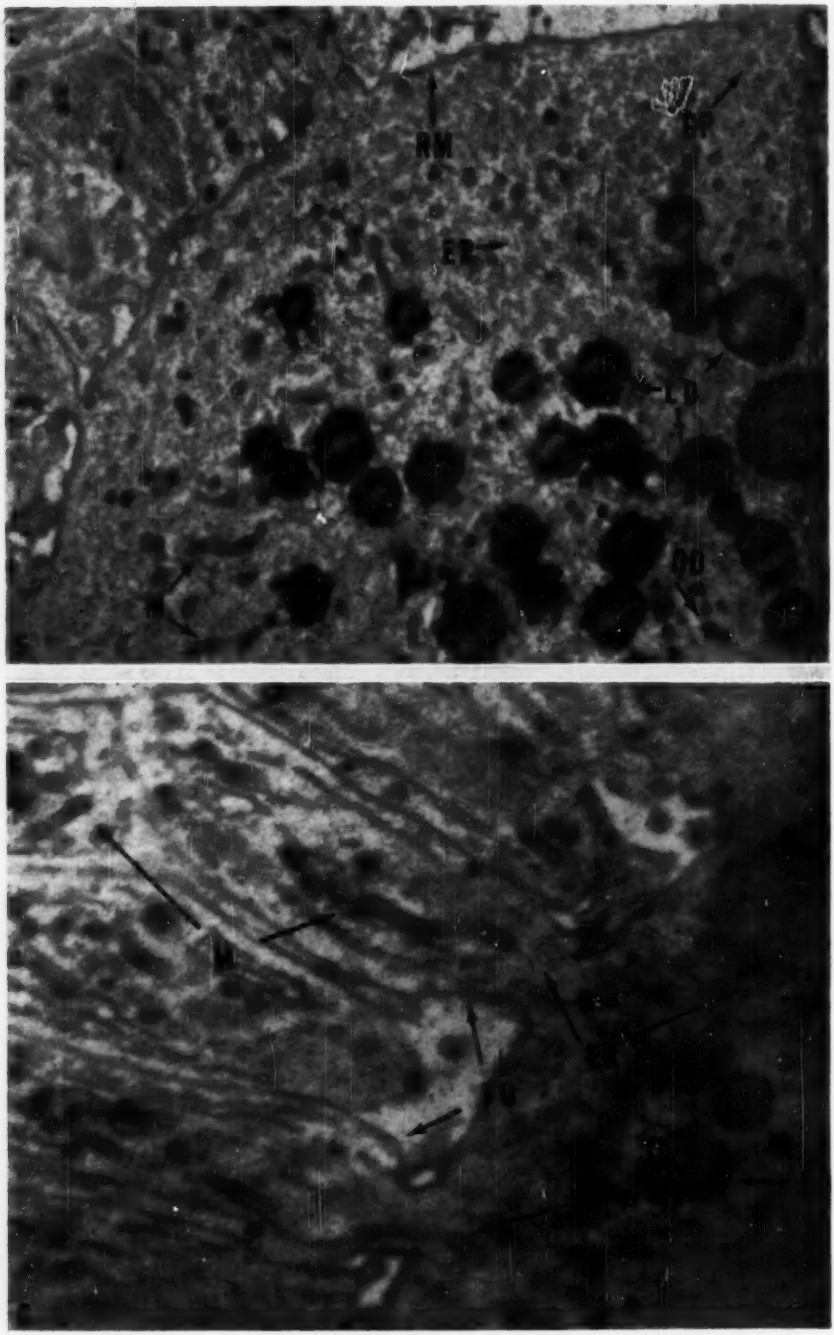


PLATE IV

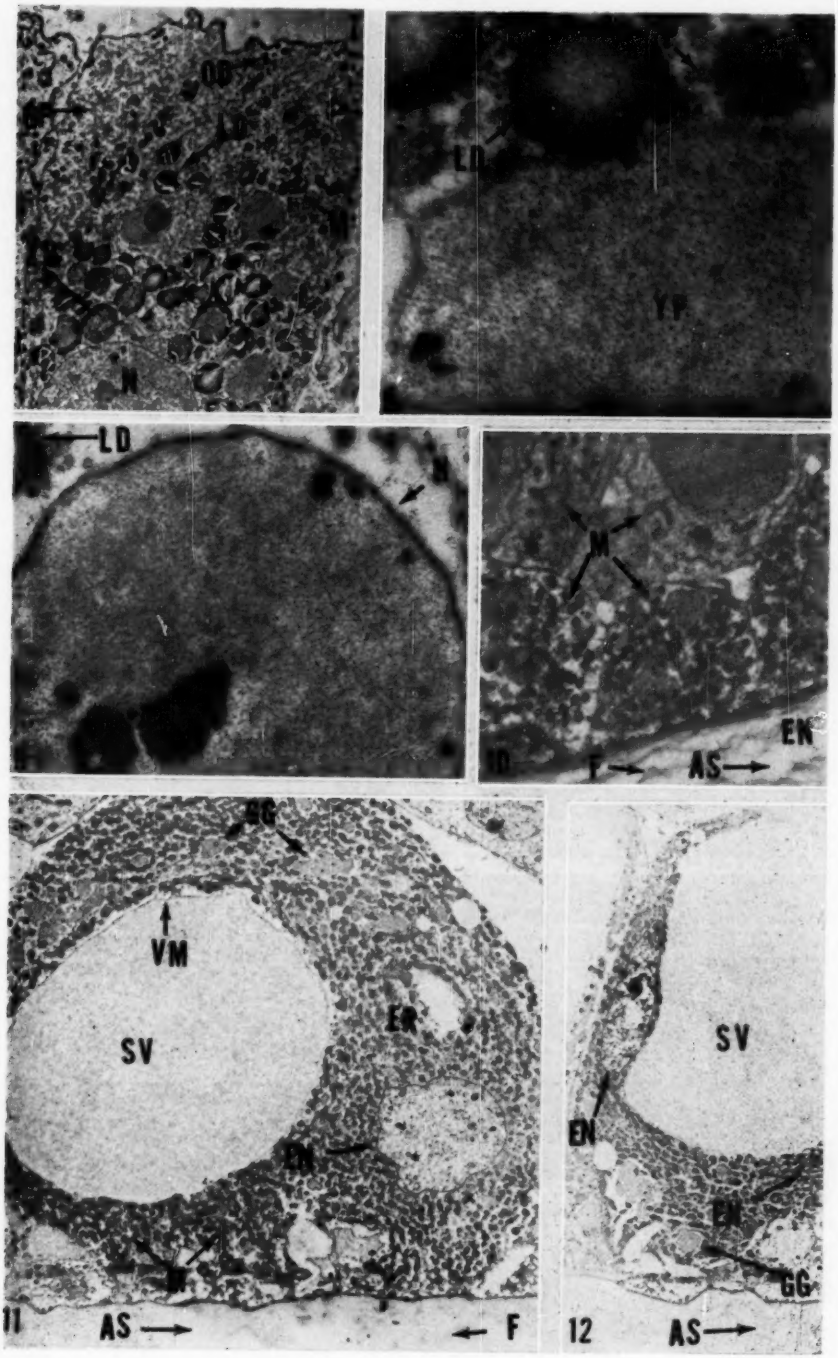
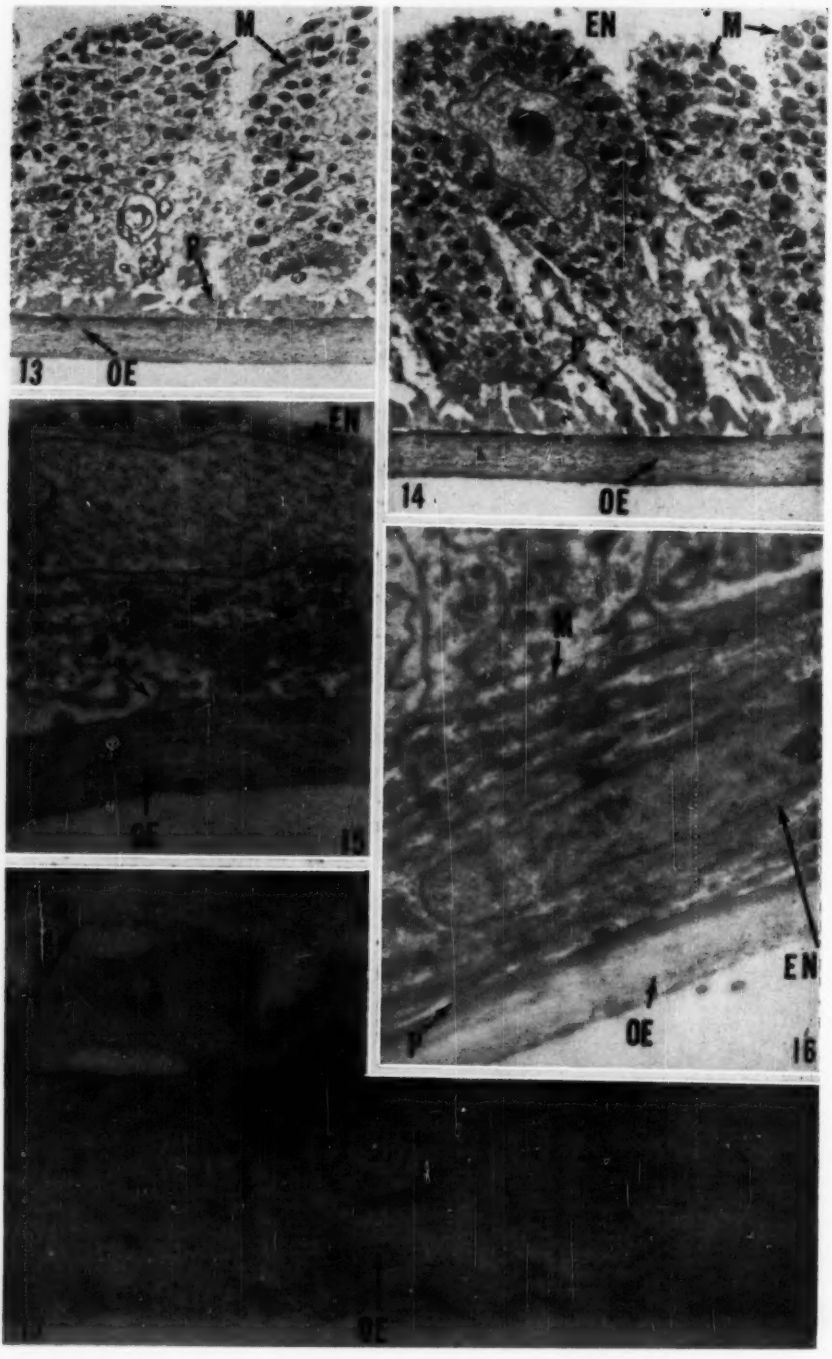


PLATE V



A CASE OF *CAPILLARIA* INFECTION CAUSING CUTANEOUS CREEPING ERUPTION IN MAN

KAORU MORISHITA AND TSUNEO TANI

Department of Parasitology, Research Institute for Microbial Diseases, Osaka University, and Surgical Clinic, Kaisei Hospital, Osaka, Japan

Among the nematode parasites of the genus *Capillaria*, the only species hitherto reported from the human being is *Capillaria hepatica*. The so-far-confirmed human cases, four in number, all deal with hepatic infection as generally encountered in natural hosts. In this paper the authors intend to report a case of cutaneous creeping eruption in man caused by a nematode of the genus *Capillaria*, emphasizing that the parasite has, so far, not been recorded from man and that the worms removed were fully matured.

OBSERVATIONS

The patient was a business man 43 years old who had lived in Osaka City for many years. At the beginning of February 1955 he first noted linear redness on the middle finger of the left hand and on the right ankle, which thereafter advanced a zigzag manner about 5 to 10 mm a day, causing severe pruritus. On February 26 the patient was seen by one of the authors (T.T.) who was able to remove a motile worm from the terminal of each lesion. After the operation the pruritus disappeared entirely. The two mature females were made available to us. They are almost complete in structure except that part of the uterus protrudes from the body in one specimen, probably an artefact caused by the operation.

The body is so slender that the anterior and posterior parts are not readily distinguishable macroscopically. It measures about 25 mm in length by 0.2 to 0.24 mm in maximum breadth which lies somewhat posterior to the middle of the posterior body. From this portion the body tapers posteriad, and suddenly becomes narrow near the posterior end, forming a short conical tail with somewhat truncated tip, while anteriorly it tapers more slowly, ending in a narrow, rounded head. There is an inconspicuous constriction, 0.12 mm broad, near the junction of the two parts of the body where the vulva is situated. In one specimen there are 4 other constrictions which may have been caused by the pressure of the host tissues during its migration, since the part of the uterus corresponding to the constriction contains a rather small number of eggs.

The cuticle possesses fine transverse striations anteriorly, whereas posteriorly it is finely longitudinally striated. So far as examination of in toto preparations in lactophenol can reveal, there is no bacillary band.

The head is round with a small cone in its center where the mouth opens. No special structure surrounds the mouth, although the cuticle of the head is somewhat dilated. Following the shallow buccal cavity, the tubular anterior part of esophagus, about 0.14 mm long, runs in a more or less bending course. The nerve ring could not be detected, probably on account of the condition of the specimens, although its

Received for publication September 24, 1957.

presence surrounding the anterior part of esophagus is suspected. The paraesophagus consists of 93 to 105 giant cells. The ratio of length of the anterior body or esophageal part to the whole body is 1 : 5.

The vulva is situated a little behind the posterior end of esophagus, about 5 mm from the head, and is provided with no special appendages. The uterus, occupying a large area of the body, is replete with eggs which are short elliptical in shape, measuring 0.056 to 0.068 mm by 0.037 to 0.042 mm. The egg shell is thick and of hyaline appearance with no surface pattern, and the poles, where the shell becomes thicker, are provided with prominent plugs which, however, do not project externally. The eggs in the uterus near the vulva contain fully developed embryos. Some eggs are seen which, mixing with some substance probably derived from the host tissues, adhere to the surface of the worm, revealing that they may have been laid during the migration in the host's body. The anus opens at the end of the truncated tail.

The present case involves two main problems: First, how the infection occurred; second, the taxonomic position of the parasite.

As was previously noted, the patient was a clerk engaged in a sedentary job. He had not walked barefoot on the ground for a long time, except on one occasion when he visited Hot Springs, Arima, not far from Osaka City, in January of the same year, on which occasion he entered a swamp. However, so far as present knowledge concerning the manner of infection with *Capillaria* extends, there is no reasonable base on which to premise infection with this parasite by so doing. On the contrary, the most probable route of the infection would be the chance swallowing of mature eggs, although at the present time the natural or reservoir hosts of this parasite are not yet known. Thus is seems too early to attempt a full explanation to this problem.

As to the taxonomic position of the parasite, there is no question in referring it to a species of the genus *Capillaria* sensu lato. Consequently, the problem is about its specific position. In Japan, at least 22 species of this genus have, so far, been recorded, and among them 8 are known from mammals; e.g., *C. hepatica* (Bancroft, 1893), *C. bacillata* (Eberth, 1863), *C. bovis* (Schnyder, 1906), *C. muris* (Uyeyama, 1928), *C. multicellularis* Yamaguti, 1941, *C. pipistrelli* Yamaguti, 1941, *C. suis* Yamaguti, 1943, and *C.* sp. of Yamaguti (1935). Since the first-named species, *C. hepatica,* is quite common in rodents in this country, and since there are some records abroad of it in human infections, it might be reasonable to consider its relationship to the present case. A morphological comparison, however, has readily shown a definite difference between these two species. Other species described from Japan, including those from mammals, birds, and coldblooded animals, also differ distinctly from the present species. In the literatures from abroad, however, is a paper by Swift, Boots, and Miller (1922) that was called to the authors' attention. They found a nematode parasite in subcutaneous tissues of experimental monkeys (*Macacus rhesus*) causing nodules, edema, and blisters on the palms and soles, and names it *Trichosoma cutaneum.* The characters of this nematode were briefly as follows.

The females, found principally in the blisters, measured 22 to 24 mm in length and 0.1 to 0.11 mm across the posterior body. Beginning about the middle of the body were seen numerous highly refractile fine papillae, arranged in 2 broad bands towards the posterior portion of the body. The paraesophagus consisted of

a single chain of from 85 to 95 cells. The ratio of length of the anterior part to the whole body was 1 : 6. The posterior end of the body was bluntly conical and the anus was terminal. The vulva was situated just posterior to the termination of the cuboidal esophageal cells. The egg was lemon-shaped with a thick brown shell, measuring 0.067 to 0.07 mm by 0.04 to 0.042 mm, and contained a coiled embryo.

Since they could not find the male worms, whose characters were thought to be important for determining the generic position (for *Capillaria* or *Hepaticola,* as separated at that time), they referred it provisionally to *Trichosoma,* a collective genus, as mentioned above. Later Freitas and Lent (1935) listed it in their list of the mammalian Capillarinae as *Capillaria cutanea* (Swift, Boots, and Miller, 1922)*.

Having compared the present worm with that species, the authors were at first impressed with the resemblances they show in several important respects, especially in the size of body, position of vulva, form of tail, size and form of the eggs, and the number of giant cells of the paraesophagus. However, the difference in the ratio of length of the anterior part to whole body in both species, being 1 : 5 and 1 : 6, respectively, and other points, including the fact that it caused creeping eruption in man, seem to suggest that the present worm may not be identical with *Capillaria cutanea* of Swift et al. Furthermore, it might appear rather reasonable to refer it to a new species. The authors, however, would like to postpone such a procedure at present, because the male, whose characters are very important for taxonomic determination, is not yet known. The diagnostic characters of the female worm only are given below.

Capillaria sp. from man

Female: With very slender body, measuring about 25 mm long and 0.2 to 0.24 mm in maximum breadth. Becillary band not observed. Paraesophageal cells 93 to 105 in number. Ratio of length of anterior part to whole body 1 : 5. Vulva a little behind the posterior end of esophagus, with no special appendages. An inconspicuous constriction at the vulvar level. Tail conical with somewhat truncated end. Anus terminal. Eggs short elliptical, thick-shelled, with smooth surface, measuring 0.056 to 0.068 mm by 0.037 to 0.042 mm, and embryonated when laid.

Male: Unknown.

Habitat: Cutaneous tissue of man.

Locality: Japan.

DISCUSSION

Although the present species is thought to differ from *Capillaria cutanea* of Swift et al, it is notable in that it shows a peculiar similarity in occurring in the cutaneous tissue of mammals, causing subcutaneous nodules, edema, and blisters in monkeys or creeping eruption in man, a phenomenon which is not known in other members of the genus. In possible relation to their finding, Swift et al cited Castellani and Chalmers' report on a skin disease, dermatitis macrogyrata, found rarely in the natives of Ceylon and South India, whose etiology is still unclarified, but which resembles closely the disease they had seen in monkeys. They were led to suggest that the two diseases had a common cause. The present authors are of the opinion, even if the conjecture of Swift et al is accepted, that the present case differs from the disease mentioned above, both clinically and parasitologically. It

* Note by the Editor: According to a reviewer, this species is now known as *Anatrichosoma cutaneum* (Swift, Boots, and Miller, 1924) Chitwood and Smith, 1958 (Proc. Helm. Soc. Wash. **25**: 112-117.)

is true, notwithstanding, that there is a group among the nematodes of the genus *Capillaria* which, instead of lodging in the alimentary canal or related organs, occurs in cutaneous tissues, causing various skin lesions.

It is well known that some parasitic nematodes, after entering unsuitable hosts, may migrate in the host's body maintaining, as a rule, the larval form, although there are known in Japan some exceptions in gnathostomiasis of man in which the mature or nearly mature worm, principally the male, is occasionally found migrating. Thus arise questions related to the present case: Is the occurrence of the worm observed in man natural or accidental? if only accidental, what animal is the natural host? when and how did the female found in the present case copulate with the undetected male? No data, however, are so far available for solving these problems.

SUMMARY

(1) A nematode parasite of the genus *Capillaria* causing typical cutaneous creeping eruption on the middle finger of the left hand and on the right ankle of a male Japanese is described. It is a parasite hitherto unrecorded from human beings.

(2) The material consists of two mature females with uteri replete with eggs containing coiled embryos when laid.

(3) Although the parasite shows a close morphological resemblance to *Capillaria cutanea,* described by Swift, Boots, and Miller (1922) from monkeys and causing subcutaneous nodules, edema, and blisters, some important characters make the authors hesitate to recognize their specimens as being identical; furthermore, it seems to be reasonable to refer the present worm to a new species. However, the authors prefer to forego its specific determination until the male worm is found.

(4) There remain several problems to be solved regarding the parasite: among them, how the host became infected and the behavior of the worm in the human body after entering it.

(5) The following points are emphasized: A new parasite has been added to the list of human parasites; a new causative agent of creeping eruption in man has been proposed; the adult worm, contrary to general knowledge concerning *Capillaria* sensu lato, has been found migrating in the cutaneous tissue.

LITERATURE CITED

FREITAS, J. F. DA T. AND LENT, H. 1935 Etudo sobre os Capillarinae parasitos de mammiferos (Nematoda, Trichuroidea). Mem. Inst. Osw. Cruz **31:** 86–160.

SWIFT, H. F., BOOTS, R. H. AND MILLER, C. P. 1922 A cutaneous nematode infection in monkeys. J. Exp. Med. **35:** 599–620.

EXPLANATION OF PLATE

Photographs of *Capillaria* sp. from man in lactophenol mounts.

FIGURE 1. Whole body of a female worm with uterus partly protruding from the body. ×4.

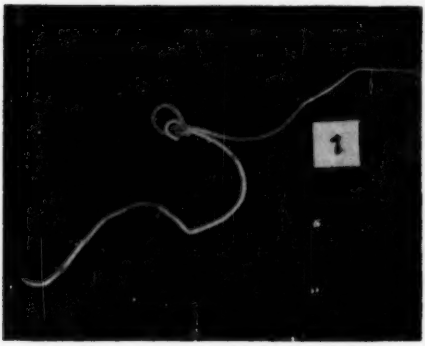
FIGURE 2. Anterior part of body, showing head with dilated cuticle and anterior part of esophagus followed by paresophagus. ×270.

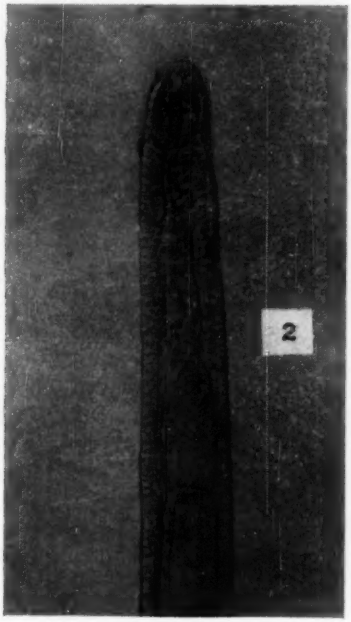
FIGURE 3. Eggs outside the body. ×240.

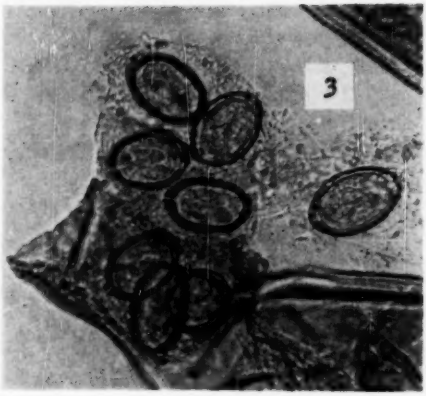
FIGURE 4. Portion at junction of anterior and posterior parts of body, showing a constriction and opening of vulva (v). ×195.

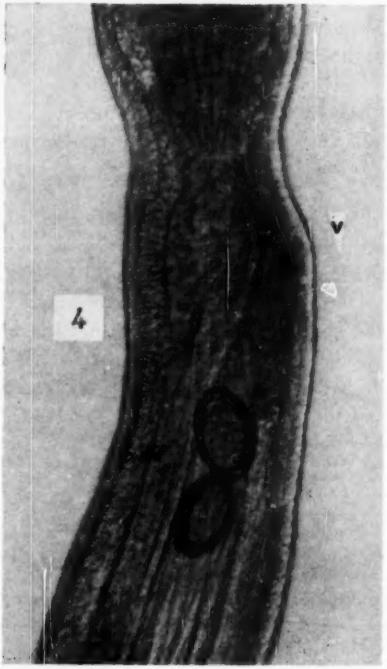
FIGURE 5. Tail part with truncated end where anus opens. ×83.

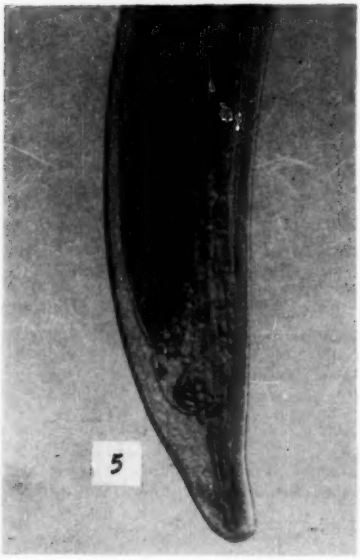
PLATE I











SOME HEMIURID TREMATODES OF OREGON MARINE FISHES*

JAMES E. MCCAULEY
Oregon State College, Corvallis

In a survey of the trematode parasites of marine fishes from the vicinity of Newport, Oregon, 156 fish representing 34 species from Yaquina Bay and the Pacific Ocean near the mouth of the bay were examined. Among the trematodes found were eight species which belong in the family Hemiuridae as restricted by Yamaguti (1958). He recognizes some but not all the families that have been proposed by various investigators to include many of the trematodes that would belong in the family Hemiuridae in the more inclusive sense of earlier studies.

Marine fishes of the West Coast of the continental United States have not been extensively examined for trematodes and hemiurids were unknown there until Sleggs (1927) reported one from Southern California. They have been reported since by McFarlane (1936), Lloyd (1938), Acena (1941, 1947), Margolis (1956, 1958), and Margolis and Adams (1956) from Washington and British Columbia; by Park (1936), Annereaux (1947), Manter and VanCleave (1951), Johnson and Copsey (1953), and Montgomery (1957), from California; but only by Gregoire and Pratt (1952) from Oregon.

All the species included in the study except *Derogenes crassus* have been previously found in fishes from the West Coast of North America. However, several new host records are made and partial resolution of certain taxonomic problems is afforded by the rather large number of individuals representing some species.

Specimens were removed from the fish, washed, flattened slightly under a coverslip, fixed with Lavdowsky's solution, and stained with Mayer's carmalum. Representatives of each species are in the Parasitological Collection of the Zoology Department of Oregon State College and the remaining specimens are in the author's collection.

Appreciation is extended to Dr. Ivan Pratt of the Zoology Department of Oregon State College for supervision of the work, aid in collecting the parasites, and critical reading of the typescript.

1. *Derogenes crassus* Manter, 1934

Dawes (1946, 1947) considered this species to be a synonym of *D. varicus*. My specimens, from 2 bottom fishes, show a more rounded posterior end, more convoluted intestinal crura, and have larger eggs (61 to 76 by 31 to 38 microns) than *D. varicus*. These characteristics, which Manter (1934) considered typical of the species, appeared in all my specimens. I agree with Manter (1947) that the species is valid. This trematode has been reported from Florida (Manter, 1934), Japan (Yamaguti, 1938), and Tasmania (Crowcroft, 1946). In Oregon 4 individuals were found in 1 *Sebastodes paucispinus* (Ayres) and 1 in *Ophiodon elongatus* Girard, thus extending the known range of *D. crassus* to the Eastern Pacific.

Received for publication April 27, 1959.

* From a thesis submitted to the Department of Zoology, Oregon State College, in partial fulfillment of the requirements for the doctor of philosophy.

Published with the approval of the Monographs Publications Committee, Oregon State College, as Research Paper No. 362, School of Science, Department of Zoology.

2. *Derogenes varicus* (Mueller, 1784) Looss, 1901

D. varicus is one of the most widely distributed trematodes and has been reported from 117 host species (Looss, 1901; Stafford, 1904; Tosh, 1905; Nicoll, 1915; Manter, 1926, 1934, 1940, 1954; Issaitschikow, 1928; Linton, 1940; Dogiel and Rosova, 1941; Miller, 1941; Heller, 1949; Rees, 1953; Yamaguti, 1958; Schullman and Schullman-Albova, 1953; Szidat, 1955; and Poljansky, 1955). Manter (1954) states that it is found in the cooler waters of the world (deeper in the tropics), and that only a few individuals are found in each host. This worm has been reported from Puget Sound, Washington (Lloyd, 1938). In Oregon 1 individual was found in 1 of 6 tomcod, *Microgadus proximus* (Girard).

3. *Genolinea laticauda* Manter, 1925

Synonym: *Genolinea robusta* Lloyd, 1938 (Manter, 1954)

This species has been previously reported from Maine (Manter, 1925), British Columbia (McFarlane, 1936), and Washington (Lloyd, 1938). In Oregon 8 individuals were collected from one *Leptocottus armatus* Girard, 16 from one *Enophrys bison* (Girard), and 2 from a *Blepsias cirrhosis* (Pallas).

Skrjabin and Guschanskaja (1954) include *Genolinea* in the family Halipegidae and renamed *Genarches* sp. Linton, 1940, *Genolinea lintoni*. I have examined Linton's specimen and description and cannot discern features that justify placing the species in *Genolinea*. It must remain *incertae sedis*.

4. *Genolinea manteri* Lloyd, 1938

This species, described by Lloyd from Seattle, Washington, has not been reported since. Six individuals were found in 1 *Lumpenus anguillaris* (Pallas) and 47 from 1 *Enophrys bison* from Oregon.

5. *Genolinea montereyensis* Annereaux, 1947

This species, described from a single individual from *Clinoccottus analis* (Girard) from Monterey, California, has not been reported since. Sixteen specimens were taken from 1 *Leptocottus armatus* from Yaquina Bay, Oregon.

6. *Hemiurus levinseni* Odhner, 1905

This widely distributed species has been reported from the European Arctic (Odhner, 1905; Issaitschikow, 1928; Schullman and Schullman-Albova, 1953; Layman, 1930; and Poljansky, 1955), England (Nicoll, 1915), the Atlantic Coast of North America (Cooper, 1915; Manter, 1925, 1926; Linton, 1940; and Heller, 1949), and Puget Sound, Washington (Lloyd, 1938). Four of 6 tomcod, *Microgadus proximus*, from Oregon yielded 17 specimens of *H. levinseni*.

7. *Lecithochirium exodicum* McFarlane, 1936

Synonyms: *Sterrhurus magnatestis* Park, 1936
Lecithochirium medium Acena, 1941
Sterrhurus exodicus (McFarlane, 1936) Yamaguti, 1958
Adinosoma exodica (McFarlane, 1936) Skrjabin and Guschanskaja, 1955
Dissosaccus medius (Acena, 1941) Skrjabin and Guschanskaja, 1955
Lecithochirium magnatestis (Park, 1936) Skrjabin and Guschanskaja, 1955

The genera *Lecithochirium* Lühe, 1901, and *Sterrhurus* Looss, 1907, are closely related and much confusion has arisen in attempts to separate them.

Yamaguti (1958) lists 30 species of *Lecithochirium* and 20 species of *Sterrhurus*, apparently accepting Crowcroft's (1946) distinction on the basis of an ejaculatory vesicle in *Sterrhurus* and its absence in *Lecithochirium*. Manter (1954) tentatively separates them by the presence in *Lecithochirium* of a pre-acetabular pit which is lacking in *Sterrhurus*. He suggests that allocation of species to those genera be deferred until they are thoroughly restudied. Skrjabin and Guschanskaja (1955) have revised the entire group, apparently eliminating the genus *Sterrhurus* by distributing its species among several other genera. That action may solve part of the taxonomic problem, but their treatment of *Lecithochirium exodicum*, as I interpret it, is indeed strange.

One species representing the *Lecithochirium-Sterrhurus* group was found in this study and is identified as *Lecithochirium exodicum*. It apparently is restricted to the Pacific Coast of North America as it has been reported thus far only from British Columbia (McFarlane, 1936), Puget Sound, Washington (Lloyd, 1938, Acena, 1941), Coos Bay, Oregon (Gregoire and Pratt, 1952), and Dillon Beach, California (Park, 1936). In the vicinity of Newport, Oregon, 10 individuals were taken from 2 *Ophiodon elongatus* brought in by commercial fishermen. These forms confirm the synonymy given above and suggested by Manter (1947). The recognition of 3 distinct species and their allocation to as many genera by Skrjabin and Guschanskaja (1955) thus is to be rejected.

8. *Tubulovesicula lindbergi* (Layman, 1930) Yamaguti, 1934

Among the species here considered to be synonyms of *T. lindbergi; T. californica* and *T. spari* were each described from 1 specimen; the latter was subsequently emended to include data from a second individual (Yamaguti, 1938). *T. madurensis* was described from 4 specimens and *T. muraenosocis* from 8 with 3 added later (Yamaguti, 1938). *T. pseudorhombi* was described from an undisclosed number of specimens (at least 2) from 1 host, as were both *T. nanaimoensis* and *T. lindbergi*, except that several host species were involved. My collection of 82 individuals from 8 host species showed considerable variation in the characters that have been used to separate the above species. A single specimen of *Leptocottus armatus* yielded 55 worms which could be placed in a graded series from which it was obvious that they represented but a single species. Yet, individual worms could be keyed to 6 of the species in Nigrelli's (1940) key: *T. spari, T. muraenosocis, T. californica, T. madurensis, T. pseudorhombi,* and *T. lindbergi*. Thirteen of my specimens would not fit the key and could be separated into 6 additional "species" on the basis of characteristics used in Nigrelli's key. Furthermore the description of *T. nanaimoensis* is so broad as to include most of my specimens.

Variations in the degree to which the prostate gland cells enclose the prostatic vesicle can be explained in terms of development; in younger worms, as judged by their having only a few ova, the posterior end of the prostatic vesicle often was not so enclosed whereas gland cells surrounded the entire vesicle in more mature individuals.

I found the 9 following patterns of the vitelline lobes in my specimens of *T. lindbergi*, expressed as the number of lobes on the right and left sides, respectively: 3-3, 3-4, 4-3, 4-4, 4-5, 5-4, 5-3, 3-5, and 6-3. Manter (1954) found variation in that feature of his material, but indicated that there were always 7 or 8 lobes. I agree with him that the vitelline pattern is of little value in separating species of

Tubulovesicula. He also emphasized that age, degree of contraction, etc., may alter some of the characters used for that purpose.

The following redescription of the species seems desirable to include the variations shown in my material and simplify the taxonomy of the genus:

Tubulovesicula lindbergi (Layman, 1930) Yamaguti, 1934

Synonyms: *Lecithaster lindbergi* Layman, 1930
Lecithurus lindbergi (Layman, 1930) Piguelewsky, 1938
Dinurus nanaimoensis McFarlane, 1936
Tubulovesicula spari Yamaguti, 1934
T. muraenosocis Yamaguti, 1934
T. californica Park, 1938
T. pseudorhombi Yamaguti, 1938
T. madurensis Nigrelli, 1940
T. nanaimoensis (McFarlane, 1936) Manter, 1947

With characters of the genus. Body spindle-shaped, often with ends sharply attenuated but occasionally with anterior end broadly rounded; 1.10 to 3.80 mm long exclusive of ecsoma, 0.30–1.37 mm wide. Ecsoma prominent, extensible to varying degrees, maximum observed 1.60 mm (body length 2.10 mm). Oral sucker sub-terminal, directed ventrally, 0.10 to 0.35 by 0.10 to 0.38 mm. Ventral sucker about ¼ body length from anterior end, 0.23 to 0.38 mm in diameter. Genital pore at level of pharynx. Excretory pore at tip of ecsoma. Cuticle smooth, unarmed. No pre-acetabular pit. Pharynx globular, close behind oral sucker, 0.08 to 0.12 mm in diameter. Esophagus short, intestinal crura usually extend well into ecsoma; sphincter muscles at junction of esophagus and each cecum can function independently. Testes spherical or ovoid, slightly behind ventral sucker, well separated, symmetrical, 0.12 to 0.20 by 0.10 to 0.30 mm. Seminal vesicle tubular, sinuous, at about level of ventral sucker. Prostatic vesicle tubular, long, with some convolutions, partially or completely surrounded with prostate gland cells; its posterior end usually, though not always, within the zone occupied by ventral sucker. Sinus sac pyriform, with strong muscular sheath; sac traversed by hermaphroditic duct formed by junction of prostatic vesicle and metraterm at base of sac; duct at first enlarged then narrowing as it approaches genital pore. Ovary 0.14 to 0.35 mm in diameter, close posterior to testes, generally more ventral and medial than testes. Oviduct runs posteroventrally from ovary to Mehlis' gland. Common vitelline duct enters oviduct in Mehlis' gland. Vitellaria of 6 to 9, usually 7, digitate lobes arising from distal ends of short vitelline ducts; usually at least 3 lobes on each side, highly variable in arrangement. Uterus with ascending and descending arms, much coiled, and filled with ova measuring 18 to 23 by 12 to 22 microns. Excretory bladder Y-shaped, bifurcating at level of testes, arms uniting dorsal to oral sucker. Parasites of stomach of marine fishes.

Hosts and localities: Pleuronectidae and other marine fishes, Peter the Great Bay (*L. lindbergi* Layman, 1930); *Sparus macrocephalus, Pagrosomus unicolor,* Japan, (*T. spari* Yamaguti, 1934, 1938); *Muraenesox cinereus, Trachinocephalus myops, Lophius litulon, Inimicus japonicus, Congor myriaster,* and *C. nystrom,* Japan; *Muraenesox coniceps,* Panama, (*T. muraenosocis* Yamaguti, 1958); *Scorpaena madurensis,* Madeira Island, (*T. madurensis* Nigrelli, 1940); *Pseudorhombus pentophthalmus,* Japan, (*T. pseudorhombi* Yamaguti, 1938), *Enophrys bison,* California, (*T. californica* Park, 1936); *Parophrys vetulus, Scorpaenichthys marmoratus,* British Columbia, (*L. nanaimoensis* McFarlane, 1936); *Leptocottus armatus, Psettichthys melanostictus, Platyichthys stellatus, Citharichthys sordidus, C. stigmaeus, Anoplarches purpurescens, Enophrys bison, Ophiodon elongatus,* Newport, Oregon; *Oncorhynchus tschawytscha,* Alsea Bay, Oregon; *Lepidopsetta bilineata,* Netarts Bay, Oregon; *Psettichthys melanostictus,* Puget Sound, Washington.

SUMMARY

Derogenes crassus, D. varicus, Genolinea laticauda, G. manteri, G. montereyensis, Hemiurus levinseni, Lecithochirium exodicum, and *Tubulovesicula lindbergi* are reported from fishes from the vicinity of Newport, Oregon, and all have new host records. *Genolinea lintoni* (Skrjabin and Guschanskaja, 1954), is considered incertae sedis. *Lecithochirium magnatestis* (S. and G., 1955), *Adinosoma exodica* (S. and G., 1955), and *Dissosaccus medius* (S. and G., 1955) are rejected. A large collection of *Tubulovesicula lindbergi* shows variations that reduce *T. spari, T.*

muraenosocis, T. californica, T. pseudorhombi, T. madurensis, T. nanaimoensis, Dinurus nanaimoensis, Lecithurus lindbergi, and *Lecithaster lindbergi* to synonymy. In *Tubulovesicula* the degree to which prostate gland cells enclose the prostatic vesicle appears to be a function of maturity and not a taxonomic character.

Literature Cited

Acena, S. P. 1941 Preliminary notes on a trematode with two yolk reservoirs and a new species of *Lecithochirium*. Phil. J. Sci. **75:** 285–289.

———1947 New trematodes from Puget Sound fishes. Tr. Am. Micr. Soc. **66:** 127–139.

Annereaux, R. F. 1947 Three new trematodes from marine fishes of California. Tr. Am. Micr. Soc. **66:** 249–255.

Cooper, A. R. 1915 Trematodes from marine and fresh-water fishes including one species of ectoparasitic turbellarian. Tr. Roy. Soc. Can. Sec. 4, Ser. 3 **9:** 181–205.

Crowcroft, P. W. 1946 A description of *Sterrhurus macrorchis* n. sp. with notes on the taxonomy of the genus *Sterrhurus* Looss (Trematoda-Hemiuridae). Pap. and Proc. Roy. Soc. Tasmania 1945: 39–47.

Dawes, B. 1946 The Trematoda: With Special Reference to British and Other European Forms. Cambridge Univ. Press.

———1947 The Trematoda of British Fishes. The Ray Society, London.

Dogiel, V. and Rozova, A. 1941 Die Parasiten des *Myoxocephalus quadricornis* in verschiedenen Gebieten seiner Verbreitung. Uchen. Zapiski Leningrad Gosudarst, Univ. Ser. Biol. Nauk (18), pp. 4–19.

Gregoire, E. and Pratt, I. 1952 Helminth parasites of the petrale sole. J. Parasit. **38:** 84.

Heller, A. F. 1949 Parasites of cod and other marine fishes from the Baie de Chaleur region. Can J. Res., Sec. D **27:** 234–264.

Issaitschikow, I. M. 1928 [Zur Kenntniss der parasitischen Wurmer einiger Gruppen von Wirbeltieren der russichen Arktis.] Trudy Morsk. Nauk Inst. Moskova **3:** 5–79.

Johnson, W. F. and Copsey, J. E. 1953 *Opisthadena bodegensis* n. sp. (Trematoda) from Dillon Beach, California. Tr. Am. Micr. Soc. **72:** 78–81.

Layman, E. M. 1930 [Parasitic worms from the fishes of Peter the Great Bay.] Izvest. Tikhookaensk. Nauchno-Prom. Stantsii, Vladivostok **3:** 1–120.

Linton, E. 1940 Trematodes from fishes mainly from the Woods Hole region, Massachusetts. Proc. U. S. Natl. Mus. **88:** 1–172.

Lloyd, L. C. 1938 Some digenetic trematodes from Puget Sound fish. J. Parasit. **24:** 103–133.

Looss, A. 1901 Über einige Distomen der Labriden des Triester Hafens. Cbl. Bakt. I **29:** 398–405, 437–442.

McFarlane, S. H. 1936 A study of the endoparasitic trematodes from marine fishes of Departure Bay, B. C. J. Biol. Bd. Can. **2:** 335–347.

Manter, H. W. 1925 Some marine trematodes of Maine. J. Parasit. **12:** 11–18.

———1926 Some North American fish trematodes. Ill. Biol. Monog. **10:** 127–364.

———1934 Some digenetic trematodes from deep-water fish of Tortugas, Florida. Carnegie Inst. Wash., Pap. Tortugas Lab. **28:** 257–345.

———1940 Digenetic trematodes of fishes from the Galapagos Islands and the neighboring Pacific. Rept. Allan Hancock Pac. Exped. **2:** 325–497.

———1947 The digenetic trematodes of marine fishes of Tortugas, Florida. Am. Midl. Nat. **38:** 257–416.

———1954 Some digenetic trematodes from fishes of New Zealand. Tr. Roy. Soc. New Zealand **82:** 475–568.

Manter, H. W. and Van Cleave, H. J. 1951 Some digenetic trematodes, including eight new species, from marine fishes of La Jolla, California. Proc. U. S. Nat. Mus. **101:** 315–342.

Margolis, L. 1956 Anomalous development of vitellaria in *Hemiurus levinseni* (Trematoda). Can. J. Zool. **34:** 207–208.

———1958 A new species of *Lecithophyllum* from North Pacific fishes with a consideration of the taxonomy of the genera *Lecithophyllum, Aponurus,* and *Brachadena* (Trematoda: Hemiuridae). Can. J. Zool. **36:** 893–904.

Margolis, L. and Adams, J. R. 1956 Description of *Genolinea oncorhynchi* n. sp. (Trematoda: Hemiuridae) from *Oncorhynchus gorbuscha* in British Columbia with notes on the genus. Can. J. Zool. **34:** 573–577.

Miller, M. J. 1941 A critical study of Stafford's report on "Trematodes of Canadian fishes" based on his trematode collection. Can. J. Res. **19:** 28–52.

Montgomery, W. R. 1957 Studies on digenetic trematodes from marine fishes of La Jolla, California. Tr. Am. Micr. Soc. **76:** 13–36.

NICOLL, W. 1915 A list of trematode parasites of British marine fish. Parasitology **7:** 339–378.
NIGRELLI, R. F. 1940 Two new species of trematode from the deep sea scorpion fish, *Scorpaena madurensis* Cuv. and Val. Zoologica: Sci. Contr. N. Y. Zool. Soc. **25:** 264–268.
ODHNER, T. 1905 Die trematoden des arktischen Gebiets. Fauna Arctica (Jena) **2:** 291–372.
PARK, J. T. 1936 Two new trematodes, *Sterrhurus magnatestis* and *Tubulovesicula californica* (Hemiuridae) from littoral fishes at Dillon's Beach, California. Tr. Am. Micr. Soc. **55:** 477–482.
POLJANSKY, I. I. 1955 [Materials from parasitic fauna of fish of the north seas of the USSR. Parasites of fish from the Barents Sea.] Trudy Zool., Acad. Nauk. SSSR **19:** 1–170.
REES, F. G. 1953 Some parasitic worms of fishes off the coast of Iceland. Parasitology **43:** 15–26.
SHULLMAN, S. S. AND SHULLMAN-ALBOVA, R. E. 1943 [Parasites of fish of the White Sea.] Izd-vo, Acad. Nauk. SSSR, pp. 51–52.
SKRJABIN, K. I. AND GUSCHANSKAJA, L. X. 1954 [Suborder Hemiurata (Markevitsch, 1951)] *in* Skrjabin, K. I. [Parasites of Animals and Man], Moskova **9:** 227–650.
———1955 [Suborder Hemiurata (Markevitsch, 1951)] *in* Skrjabin, K. I. [Parasites of Animals and Man], Moskova **10:** 339–649.
SLEGGS, G. F. 1927 Notes on cestodes and trematodes of marine fishes of Southern California. Bull. Scripps Inst. Oceanog., Tech. S **1:** 63–72.
STAFFORD, J. 1904 Trematodes from Canadian fishes. Zool. Anz. **27:** 481–495.
SZIDAT, L. 1955 La fauna de parasitos de *Merluccius hubbsi*. Comun. Inst. nac. B. Rivadavia Zool. **3:** 1–54.
TOSH, J. R. 1905 On the internal parasites of the Tweed salmon. Ann. Mag. Nat. Hist. 7. s. (92) **16:** 115–119.
YAMAGUTI, S. 1934 Studies on the Helminth Fauna of Japan 2. Trematodes of Fishes I. Jap. J. Zool. **5:** 249–541.
———1938 Studies on the Helminth Fauna of Japan 21. Trematodes of Fishes IV. Rev. ed. (published by the author). Maruzen Co. Ltd., Tokyo.
———1958 Systema Helminthum. Vol. 1. Digenetic Trematodes. Interscience Publishers, New York.

RESEARCH NOTE

PEPTIDASE AND LIPASE ACTIVITY OF EXTRACTS OF *SCHISTOSOMA MANSONI* CERCARIAE.

Extracts of cercariae of *Schistosoma mansoni* have been shown to possess extensive proteolytic activities on complex substrates of native and altered protein (Lewert and Lee, 1954, J. Inf. Dis. **95:** 18–51, 1956, J. Inf. Dis. **99:** 1–14). Mucopolysaccharidase activity is also present (Lee and Lewert, 1957, J. Inf. Dis. **101:** 287–294). In the present study, extracts of *Schistosoma mansoni* cercariae were tested for peptidase activity and were compared with extracts of *Strongyloides ratti* and *Nippostrongylus muris* for lipase activity.

One-tenth ml quantities of cercarial extract, prepared at 0 C from a homogenate of 157 mg lyophilized cercariae in 17 ml 0.85 percent saline, were added to tubes containing 0.1 ml of a 1 percent solution of glycylglycine, glycyl-L-proline, prolylglycine, L-leucylglycine, or L-leucylglycylglycine in 0.1 M Sörensen's phosphate buffer at pH 7.1, pH 7.5, or pH 7.9. Controls for the presence of free amino acids in the cercarial extract and for the purity of the peptide substrates were also prepared. The tubes were incubated at 37 C for 2 hours with continual mixing. Samples from each tube and from amino acid controls were spotted on No. 1 Whatman filter paper and ascending chromatograms were made with butanol-acetic acid-water (4:5:1) as the solvent. The chromatograms were developed by spraying with 0.2 percent ninhydrin in acetic acid.

No free amino acids were found in the cercarial extracts. Each peptide was split completely into its component amino acids by the cercarial extract, which was completely effective at each pH.

Lipolytic activities of saline extracts, prepared in the cold from 10 mg lyophilized *Schistosoma mansoni* cercariae, *Strongyloides ratti* filariform larvae or *Nippostrongylus muris* filariform larvae per ml of 0.85 percent saline, were compared to the activities of similar extracts of pancreatin and to an enzyme-free buffer control. Two-hundredths ml of 0.1 M Sörensen's phosphate buffer was mixed with 0.04 ml extract and placed on a thin film of glycerol tri-

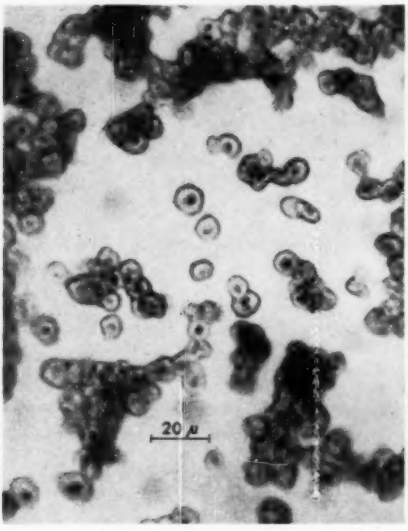


FIGURE 1. Tripalmitin in nitrocellulose film.

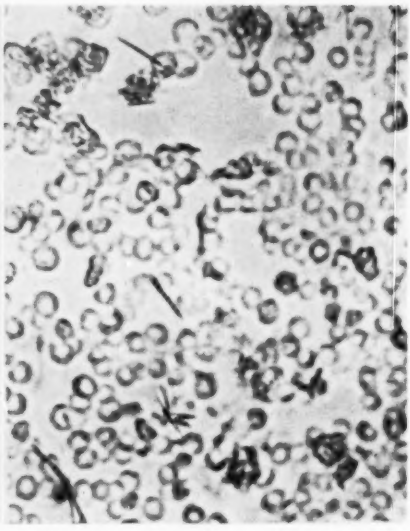


FIGURE 2. Tripalmitin and palmitic acid spicules in nitrocellulose film.

palmitate (tripalmitin) in nitrocellulose on a cover glass. The films were prepared by dipping cover glasses into a solution of 10 mg tripalmitin per ml of 0.75 percent nitrocellulose in 94 parts of ethyl ether and 6 parts of absolute alcohol. A perdominantly homogeneous dispersion of tripalmitin in a nitrocellulose matrix resulted. Each tripalmitin particle was spheroid in shape, approximately 10 microns in diameter, and appeared to be composed of a series of spheres strung together in a circle (fig. 1). The cover glasses were suspended over depression slides, ringed with paraffin to prevent evaporation of the test solution, and incubated at 37 C for 8 days. The films were examined at magnifications of 990 diameters or less, at intervals of 2 hours, 4 hours, 24 hours, 48 hours, 144 hours, 240 hours, and 384 hours from the time of preparation for the presence of clear, refractile, spicules and plaques similar to those seen in films of palmitic acid.

Optimal activity of the pancreatin occurred at pH 8.0 and pH 8.4. All the experiments with the helminth extracts were made at pH 8.0. No obvious formation of palmitic acid-like spicules was seen in the buffer control. At 2 hours, only the pancreatin showed activity. At 4 hours, and upon subsequent examination, all the helminth extracts showed some activity. After 24 hours, numerous spicules and plaques were present in all the preparations containing extract except that of *S. mansoni*. This preparation contained markedly fewer spicules. The number and size of spicules and plaques increased with time in all the preparations containing extracts. However, after 8 days of incubation, noticeably fewer spicules were seen in the *S. mansoni* preparations than in the other helminth preparations. The spicules appeared to originate from the spheroid granules of tripalmitin and were often clumped in pairs or groups of 3 or more, joined at the base, and radiating into the surrounding medium (Figure 2).

(These investigations were conducted under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and were supported in major part by the Office of the Surgeon General, Department of the Army).—S. MANDLOWITZ, D. DUSANIC, AND R. M. LEWERT, *Department of Microbiology, The University of Chicago, Chicago 37, Illinois.*

THE LIFE HISTORY OF THE SALMON-POISONING FLUKE, *NANOPHYETUS SALMINCOLA* (CHAPIN)*

ELWIN BENNINGTON
Training Officer, U.S.P.H.S. Training Branch, CDC, Atlanta, Georgia
and
IVAN PRATT
Professor of Zoology, Oregon State College, Corvallis, Oregon

The life history of *Nanophyetus* (= *Troglotrema*) *salmincola* (Chapin) has been studied by several workers who were interested chiefly in a disease of canines transmitted by this trematode (Simms, Donham and Shaw, 1931; Cordy and Gorham, 1950; and Philip et al, 1954). The etiologic agent has been considered by various workers to be distemper virus (Suckley, 1855), a bacterium (Bonebrake, 1925), an ameba (Pernot, 1911), mechanical destruction (Hoeppli, 1926), a haemosporidian or rickettsia (Simms and Muth, 1933), a rickettsia (Cordy and Gorham, 1950), and *Neorickettsia helmintheca,* Philip, Hadlow and Hughes, 1953. The role of the fluke is still not clear, but its association with salmon-poisoning disease was established in 1925 by C. R. Donham (see Donham, 1928).

Donham's fluke was named *Nanophyes salmincola* by E. A. Chapin (1926), who (1928) amended the generic name to *Nanophyetus.* This animal, along with *Nanophyetus schikhobalowi* Skrjabin and Podjapolskaja, 1931, from Siberian aborigines, was subsequently transferred to *Troglotrema* Odhner, 1914, by Witenberg (1932) who believed *T. schikhobalowi* to be conspecific with *T. salmincola.* Wallace (1933) considered *Troglotrema* for a related fluke, *Sellacotyle mustelae* Wallace, 1935, and retained the genus *Nanophyetus* for *salmincola.*

The molluscan host was believed (Simms, Donham and Shaw, 1931) to be *Oxytrema* (= *Goniobasis*) *silicula* (Gould) as a result of work and unpublished drawings of Sinitsin (Wallace, 1935). There was confusion (Donham, 1928) as to which of several cercariae was that of *N. salmincola.* Sinitsin's (1930) descriptions of the larval forms are indeed confused. The important fish hosts were known to be species of *Salvelinus, Salmo,* and *Onchorhynchus. Distomulum oregonensis,* proposed for the metacercariae by Ward and Mueller (1926) who believed them to cause pop-eye disease of trout, was relegated to synonymy (Price, 1929).

The purpose of this research was to complete the life history of *Nanophyetus salmincola* and to illustrate the various stages.

MATERIALS AND METHODS

Infected fish were obtained in the vicinity of Corvallis, Oregon, or were donated by the Oregon Game Commission Alsea Hatchery. Uninfected fish were obtained from the Oregon State Fish Commission hatchery at Camp Sherman, Oregon. Infected snails were collected from local streams.

Adult flukes were obtained from dogs, raccoons and golden hamsters which were used as

Received for publication May 3, 1959.
* Published with the approval of the Oregon State College Monographs Committee. Research Paper No. 361, Department of Zoology, School of Science.

experimental definitive hosts. Adults were sketched from living material, and cultured experimentally in hamsters. Free adults and adults in situ in intestine were fixed in either Bouin's or acetic-alcohol-formalin mixture. Hemotoxylin and eosin stains were used on sectioned material; whole flukes were stained with Mayer's carmalum.

Eggs were incubated in tall flasks at room temperature. Metacercariae were taken from wild fish and from trout experimentally infected.

Rediae and cercariae were obtained from local snails. Cercariae were stained vitally with neutral red and Nile blue sulphate. Cercarial penetration was observed in finger bowls and drawings of all stages were made from living material. In all cases, histological sections were only used to confirm observations of living material.

All measurements are recorded in millimeters and were made on living as well as fixed material.

RESULTS

Witenburg's measurements for adult *Nanophyetus salmincola* were as follows: "Length 0.8 to 1.1, width 0.3 to 0.5, oral sucker 0.15 to 0.18, pharynx 0.06, esophagus 0.06 to 0.07, ventral sucker 0.12 to 0.13, testes 0.2 to 0.3." They agree very closely with ours except that maximum length may be as much as 2.5.

The ovary lies to the right of the ventral sucker (fig. 1). The short narrow oviduct enlarges abruptly into a chamber ciliated at each end and separated from the uterus by a sphincter. This chamber contains active sperm; an enlargement proximal to the ovary serves as a sperm receptacle, and although it resembles a seminal receptacle in some fixed material, is properly a fertilization chamber (fig. 2). Laurer's canal opens from the middle non-ciliated area and the common vitelline duct opens into an enlargement near the uterine end. The non-ciliated area is very elastic, and, since there was no evidence of Melhis' gland, the area labeled ootype in figure 2 is most probably the area that accommodates the eggs as they are formed; accumulations of vitelline cells were observed here and never in the uterine tube. Laurer's canal passes dorsally, then anteriorly, to open through a sphincter on the mid-dorsal line somewhat posterior to the region of the ventral sucker. In two instances sperm, vitelline cells, and bubbles were seen to pass out the pore.

The vitellaria are composed of large follicles scattered irregularly beneath the dorsal surface and extending around toward the ventral surface. Vitelline cells collect at the junction of the two viteline ducts, a short distance posterior to the ventral sucker, and pass singly, in twos, and in threes along the common vitelline duct to the ootype where an opaque oocyte is added. Rhythmic peristaltic waves pass along the uterus from the ootype to the common genital duct.

The uterus in a young specimen is a simple U-shaped tube which extends posteriorly from the ootype and then anteriorly to the common genital duct. The uterus lies in the median dorso-ventral plane and acquires a W-shape as it fills with eggs. There are usually 5 to 16 eggs. The uterus also contains spermatozoa, some of which find their way into the ootype, and others escape when eggs pass from the genital pore.

The common genital duct opens in a simple shallow cup near the posterior border of the ventral sucker. This duct has two sphincters, one near the genital pore and the other about halfway between the genital pore and the opening of the ejaculatory duct.

The two large oblong testes are lateral to the intestinal crura and surrounded except medially by extensive groups of vitelline cells. The anterior ends of the testes lie near the posterior margin of the ventral sucker. In living worms the testes constantly change from ovoid to almost rod-shape, and are drawn forward

and backward a distance of at least half their length. The two tiny vasa efferentia join to form a common duct before entering the seminal vesicle, a large bipartite structure within the cirrus sac, lying on the left of the ventral sucker.

Sperm proceed at a rather constant rate from the seminal vesicle through a minute tubule into an ejaculatory bulb which periodically closes forcing sperm into the ejaculatory duct and through a sphincter into the very anterior end of the uterus (fig. 2). The glandular wall of the ejaculatory duct forms the pars prostatica. There is no cirrus.

The adult worm may assume any shape from a sphere to a long blunt rod. It crawls very actively about the mucosa of the host's intestine. Some are more or less buried among the villi, but some are almost completely embedded, anterior end down. There is no evidence in the sectioned material of extensive mechanical damage, although the worms often grasp and hold bits of intestinal tissue in the oral and ventral suckers. Eggs are present in the uterus in about six days.

When removed to saline solution, mature flukes usually continue to extrude a few eggs. In saline the worms move actively for 48 to 60 hours and, if mucous and intestinal debris are present, remain alive in a refrigerator at least 12 days. When moving, the part of the body between the suckers is most active, although the whole body is very motile.

N. salmincola has a long list of experimental definitive hosts including the hamster and wood rat. Adult worms are eliminated from the hamster and wood rat after a period of 51 days. Two white rats and two white mice could not be infected. The flukes occur along the whole length of the small intestine of the hamster, but are limited to the upper end of the small intestine of larger animals such as the raccoon and dog.

Eggs and miracidia. The eggs are ovoid in shape, 0.087 to 0.097 by 0.038 to 0.055, light brown in color and rather heavy, so that they sink rapidly in water. They are operculated at one end and have a very small blunt point at the other (fig. 3). Early development is very slow. Eggs collected in September and kept in bottles of water at room temperature required a minimum of 87 days to hatch. Many did not hatch until the following March and April, making a total of 185 to 200 days of incubation. Eggs failed to hatch in running tap water. Aeration of water produced no change in the rate of development.

The fully developed miracidia within the eggs are very active, moving back and forth, contracting and elongating, apparently exerting force against the operculum (fig. 4). There is no ciliary action until the operculum is opened. The newly emerged miracidia (figs. 5 and 6), 0.087 to 0.105 by 0.037, swim in characteristic, long, graceful curves. They are not attracted to host snails; miracidia were observed to bump into snails repeatedly and swim away without attempting to penetrate them.

The anatomy of miracidia was difficult to determine because of their opacity and ill-defined cell boundaries. There appears to be but one flame cell.

Rediae. Rediae of various sizes, 0.45 to almost 3, were found in each infected snail (figs. 7 and 8). The pharynx measures 0.063, the sac-like gut to 0.3. The birth pore is close to the pharynx. The smallest rediae taper to a point posteriorly and are very active. Larger ones are cylindrical and very sluggish.

No redia containing daughter rediae was found. The smallest rediae contain only a few tiny germ balls, larger ones a few cercariae, and very large, mature

rediae 74 to 76 cercariae (fig. 7). No sporocyst was identified, and in serial sections of snails exposed to miracidia no sporocysts were discovered. Rediae and cercariae were found in all tissues of the host snail, but most frequently in the digestive gland. Cercariae only were shed by the snail host. No mixed infections were found, although at least five other species of cercariae develop in the host snail in the study area.

Cercariae. The living cercariae of *Nanophyetus salmincola* measure 0.31 to 0.47 by 0.03 to 0.15 (fig. 9). The average length is 0.39; the cuticula is transparent and armed with fine backward directed spines. The tail, 0.035, is bluntly conical and also bears fine hair-like spines. The body is almost circular in cross section.

The oral sucker, 0.075 to 0.08, opens subventrally and is almost spherical. The stylet, 0.012 by 0.003, is simple and points anteriorly. From the pharynx, 0.02, a few cells trail posteriorly, but do not form a gut. The ventral sucker, 0.067, lies posterior to mid-body. Near the tail is a ventral longitudinal slit opening into a deep pit, the so-called adhesive organ with walls which appear to secrete a sticky mucous substance. The secretory cells form a large mass, the cell boundaries of which are indistinct. Conspicuous penetration glands lie antero-dorsal to the ventral sucker and appear in four clusters of 4 cells each. Each cell cluster connects to the base of the stylet by a bundle of ducts, each cell apparently having its own duct. The ducts are of irregular size and usually have a dilatation near the posterior border of the oral sucker.

A cluster of cells anterior to the excretory vesicle appear to represent genital primordia. The excretory vesicle, a large, pear-shaped, hollow sac made up of cuboidal cells, empties into a median tubule which passes posteriorly and downward to open dorsally at the base of the tail. Anteriorly at each side of the excretory vesicle is a collecting duct which could not be followed. Flame cells were observed, but their pattern was obscured by the opacity of the tissues.

The cercaria elongates and contracts rapidly, and opens and closes both suckers. The stylet is protruded at least once upon each elongation of the body. There is a more or less rhythmical contraction of the large excretory bladder, and opening and closing of the aperture of the "adhesive" organ. The tail is conical and slightly contractile so that it can, at times, almost double its length. Secretion from the "adhesive" organ was not observed. Cercariae live up to 48 hours in water.

Cercariae were shed only by snails 2.5 cm or more in length, although smaller snails were frequently infected. Cercariae are shed intermittently, often by the thousands, with tiny ropes of a mucous substance. Such exudate coming from several snails was sometimes sufficient to entangle small fish. A few cercariae seemed to be free of mucus, but most of them were bound by it.

Cercariae when suspended near fish showed no response to them but constant rhythmical contraction persists until the cercaria makes contact with the fish, when it begins to penetrate. A few were observed to get beneath the skin in 30 seconds; seldom was more than two minutes required. Once embedded, they usually rest a short time, then proceed into deeper tissue. They penetrate into blood vessels between the rays and crawl in a caterpillar-like manner into the base of the tail. They penetrate so rapidly that occasionally one would perforate the thin tails and fins of fish and emerge on the opposite surface. Cercariae released by crushing snails often cannot effect penetration. Encystment in or on anything but a fish

was never observed.

A local dermatitis was observed when cercariae penetrated the skin of salmon. Many of the tails and fins of wild fish collected from the Alsea River during the low water season were eroded and heavily damaged. In histological sections, the eroded fin rays were characterized by numerous adjacent metacercariae. This was true in areas of erosion in the gills as well as other organs of wild fish including the retina and cornea of the eyes, as observed by Ward and Mueller (1926).

The parasite is pathogenic to fish. Nine young salmon placed in an aquarium with 8 snails shedding cercariae were dead in 6 hours. In other exposures with more snails than fish, some of the fish died in 12 hours or less. When the proportion of fish to snails was increased, none died as a result of a 12-hour exposure. None of the control fish died, and snail-infested water in which fish had died was not injurious when filtered.

Donham (1928) reported that cercariae encyst only in fish belonging to the family Salmonidae. However, when exposed to infection, 2 small dace minnows died, one within 12 hours, and a small sculpin was dead the following day. Two gold fish placed in an aquarium with 4 infected snails showed distress in 7 hours and died within 11 hours; the eyes, skin and tails were covered with encysting cercariae. In these fish, the cercariae apparently do not migrate into deeper tissues as readily as they do in salmon.

Metacercariae. The cercariae penetrate fish at any point, lose their tails in the act of penetration, migrate to and through the internal organs, and encyst. The new cyst wall is thin, transparent, and easily ruptured (fig. 11). When rupture occurs, the metacercaria crawls about. It can re-encyst in a few hours, the cyst seemingly being formed by the fluke and not by the host. Cysts are occasionally formed on the skin quite outside the body of the host. Presumably older metacercariae, 0.53 by 0.28, oral sucker 0.11, ventral sucker 0.10, pharnyx 0.03, excretory bladder 0.25 by 0.18, from naturally infected fish have a tougher cyst wall and are larger in size (fig. 12). The shape is generally ovoid and the cuticle is covered with fine, retrorse spines.

As metacercariae develop, the stylet becomes smaller and is usually absent in natural infections. Testes as described by Ward and Mueller were not observed, although the anlagen are present, and the dense mass of heavily staining material (nuclei?) reported by these authors was not observed. The excretory bladder fills after formation of the cyst and gradually increases in size. Its contents make it appear white by reflected light and black by transmitted light, making infections of fish easy to detect (Shaw, 1947).

Metacercariae less than 10 days of age are not infective to hamsters. Older ones mature into adults with eggs in 6 to 7 days.

Snail. The host snail, *Oxytrema silicula* (Gould), was collected in large numbers throughout the year except for a very short period of high water in the winter. They prefer large rocks, pilings, bridges, old planks, and debris on the bottom of a stream bed, and usually do not migrate into shallow water. Millions of snails occur in most coastal streams, and most of them 2 cm or larger are infected with the larvae of some trematode. There is evidence that the incidence of infection in this snail, which lives several years, is high in relation to the number shedding cercariae, because larval development continues slowly over a long period of time.

Fish. Chinook salmon fingerling died after being exposed to cercariae of *N.*

salmincola, presumably as the result of the invasion. Fish of all species so far exposed, including 42 Chinook fingerling, have become infected, but none showed signs of exophthalmus. Three small salmon were not infected by feeding them chopped fish containing metacercariae.

DISCUSSION

Although Hoeppli (1926) attributed salmon-poisoning disease to mechanical action of the trematodes, histological sections of flukes in situ show evidence of little or no such damage. Donham noted that flukes apparently had no ill effect upon immune dogs. It is, of course, probable that the disease is caused by a rickettsia-like organism transmitted by the fluke (Cordy and Gorham, 1950; Philip, Hadlow and Hughes, 1954).

Chapin (1926) and Wallace (1935) described a seminal receptacle in the adult fluke; Witenberg (1932), however, failed to locate such a structure. In most serial sections the fertilization chamber (fig. 2), located at the ovarian end of the ootype, has the appearance of a seminal receptacle filled with sperm. This chamber is located on the right side at the level of the posterior edge of the ventral sucker, posterior to the ovary, and not antero-dorsal to the seminal vesicle as described by Chapin (1926).

Laurer's canal opens on the dorsal midline at the level of the ventral sucker, not at the level of the esophagus as described by Witenberg (1932).

Simms, Donham and Shaw (1931), keping eggs at 52° to 68° F, found them to hatch in 75 days, while in this study 87 to 200 days were required.

The most common natural definitive host, or hosts, has not been determined, but the large numbers of infected snails suggest a very common source of infection, probably racoon or mink, which eat fish and frequently defecate in or near the water.

In large rediae, the gut never extends over one-half the length of the body to reach the posterior as previously described by Sinitsin (1930). Such rediae also reach 3 mm. They never produce rediae and cercariae simultaneously, as he claimed, nor were mixed infections observed in this study. The host snail harbors many kinds of cercariae, as was pointed out by Donham (1928), Sinitsin (1930) and others, but the larva of *Nanophyetus salmincola* is not the most frequently occurring one. The cercaria which Donham reported is the larva of *Acanthatrium oregonense,* the life history of which was described by Knight and Pratt (1955). Although that cercaria does crawl over a fish, as Sinitsin observed, it does not penetrate the skin, nor do any of the other trematode larvae shed by *Oxytrema silicula* except those of *N. salmincola.*

Sinitsin (1930) confused the cercaria with the metacercaria. His description does not apply to any of the cercariae shed by *O. silicula,* but it is accurate enough to indicate the cercaria of *N. salmincola.* A mucous gland is present, much as Sinitsin described it, but it is not an adhesive organ by means of which cercariae stick to their victims. The cercariae are held in groups by strands of mucous by means of which they probably effect contact with fish.

The cercariae of *Nanophyetus* rarely, if ever, crawl on the skin of fish, and very few cercariae reach the kidney via the anal aperture. Most wild fish do have a greater number of cysts in the kidney than elsewhere, often arranged in rows (Simms, Donham and Shaw, 1931). Fish do have a renal portal blood system, and, since cercariae were observed to penetrate into and follow down blood

vessels, they most probably follow this route into the kidney.

The portal of entry is probably not the gills, as suggested by Simms, Donham and Shaw (1931), although many do encyst there; entry is related to the areas of direct contact. Hundreds of tiny penetration points can be demonstrated on the bellies of small wild fish, indicating a high incidence of penetration in that area, as well as on fins, tail, gills and skin generally.

The cercariae are able to penetrate into all the muscles of a small fish in less than a day, and such invasion is definitely pathogenic to small fish (Simms, 1932), particularly young game fish during the low-water season. The erosion of fins, tails and gills in the proximity of cysts is probably restricted to the time of cercarial migration; the metacercariae are not harmful. Metacercariae are no doubt present in salmon returning from deep sea, since some were recovered from such a salmon reportedly taken at sea.

Nanophyetus salmincola is very closely related to *Nanophyetus schikhobalowi,* as was pointed out by Witenberg (1932), and to *Sellacotyle mustelae,* as was pointed out by Wallace (1935). The three worms range in size from the smallest, *S. mustelae,* 0.335 by 0.190, to *N. schikhobalowi,* 0.580 by 0.470, to *N. salmincola,* 1.1 by 0.4. The oral suckers measure 0.1, 0.1, and 0.16, respectively, and the ventral sucker measures 0.12 in both *N. schikhobalowi* and *N. salmincola.* The pharynx of all three is very short; there is possibly none in *N. schikhobalowi.* The intestine reaches to the middle of the ventral sucker in *S. mustelae* and beyond it in the other two. The testes are proportionately about the same size and occupy the same postion in all three; the seminal vesicle is large and divided in each and none possesses a cirrus. There is no cirrus pouch in *S. mustelae,* none is described for *N. schikhobalowi* and one is present in *N. salmincola.* The ovary is of the same shape and position in each; a small seminal receptacle is described for each, but the uterus is sac-like in *S. mustelae* and a simple tube in *N. salmincola.* The eggs measure 0.06 by 0.05 in *S. mustelae,* 0.067 by 0.043 in *N. schikhobalowi,* and 0.08 by 0.05 in *N. salmincola.* In all three worms, the genital pore is a simple opening directly behind the ventral sucker. Laurer's pore opens before the ventral sucker in *S. mustelae* and behind it in *N. salmincola.* The vitellaria are the same in all three. All three worms parasitize the mucosa of the upper small intestine of mammals.

There are few differences between these trematodes. The eggs of *N. schikhobalowi* are smaller and are relatively smaller than the oral sucker. *N. schikhobalowi* parasitizes man, while there is no record of the other two in man. *S. mustelae* differs from *N. salmincola* in that it is smaller, has shorter intestinal crura, has a sac-like uterus, has no cirrus pouch, and Laurer's pore is anterior in position to that in *N. salmincola.*

S. mustelae differs from *N. salmincola* much more than does *N. schikhobalowi,* but the larvae of *N. salmincola* and *S. mustelae* are very nearly identical. The larvae are microcercous xiphidiocercariae which differ in minor detail, and rediae which are morphologically quite simlar. The cercariae of *N. salmincola* measure 0.39 by 0.09 and those of *S. mustelae* 0.26 by 0.1. The oral suckers measure 0.08 by 0.075 and 0.056 by 0.052, respectively; pharynx, 0.02 and 0.017, respectively; ventral sucker, 0.067 and 0.052, respectively; and stylets, 0.013 and 0.018, respectively. Both have armed cuticula, identical mucous glands and almost identical stylet glands. The stylet glands agree even to the ducts of four elements, each of which

form an S-curve above the stylet sac. Their behavior is very similar, except that *N. salmincola* cercariae seldom crawl on a surface and can effect a more rapid penetration of the host.

The cercariae of both species become infective after 8 to 10 days in the fish host, and mature in 5 to 6 days in the mammalian gut. The rediae are morphologically similar, but differ in that those of *S. mustelae* contain fewer developing cercariae.

The three species, *N. schikhobalowi, N. salmincola* and *S. mustelae,* are very closely related and are perhaps congeneric. Skrjabin and Podjapolskaja indicated that the main differences between *N. schikhobalowi* and *N. salmincola* were egg size and position of the genital pore. Chapin was in error concerning the position of the genital pore, which is no doubt similar in *mustelae, schikhobalowi* and *salmincola;* and proportionate sizes of oral suckers and eggs may be subject to errors due to differences in tissue reaction to fixatives. Two of the most important differences between *mustelae* and *salmincola* are presence of a cirrus pouch and shape of uterus. Although Chapin's description did not mention the cirrus pouch, Skrjabin and Podjapolskaja placed *N. schikhobalowi* in the family Heterophyidae because it lacks a cirrus pouch, notwithstanding Witenberg's statement to the contrary. Since these differences are not specifically mentioned in the description of *schikhobalowi,* there is not sufficient evidence to believe that *schikhobalowi* and *salmincola* are identical. There is no record of human parasitosis by *N. salmincola,* and until there is evidence of such infection, this fluke should not be considered a parasite of man.

The fact that the species of *Troglotrema* live in cysts and possess a cirrus should exclude *N. schikhobalowi* and *N. salmincola* from that genus. Therefore, the genus *Nanophyetus* should be retained as suggested by Wallace. This genus, however, should be redefined with respect to the position and description of the genital pore, lack of true seminal receptacle, and presence of a cirrus pouch.

SUMMARY

The major stages in the life history of *Nanophyetus salmincola* (Chapin) have been identified and described. The conflicting statements in the literature concerning biology and morphology of this fluke are discussed. All species of cercariae collected from *Oxytrema silicula* (Gould) were exposed to young salmon, with the result that the microcercous xiphidiocercariae of *N. salmincola* were found to be the only ones which penetrate fish. The fish infected were subsequently examined and metacercariae typical of *Nanophyetus salmincola* demonstrated. These metacercariae were fed to hamsters and recovered from the intestine as adult flukes which fit the description of *Nanophyetus salmincola* (Chapin).

REFERENCES CITED

BONEBRAKE, A. 1925 Are salmon poisoning and distemper the same disease? Outdoor Life **55:** 418–419.

CHAPIN, E. A. 1926 A new genus and species of trematode, the probable cause of salmon-poisoning in dogs. N. Am. Vet. **7:** 36–37.

———1928 Note. J. Parasit. **14:** 60.

CORDY, D. R. AND GORHAM, J. R. 1950 The pathology and etiology of salmon disease in the dog and fox. Am. J. Pathol. **26:** 617–637.

DONHAM, C. R. 1928 Salmon poisoning in dogs. Thesis. Oreg. State Coll. Library.

HOEPPLI, R. 1926 Anatomische veranderungen des Hundarms, hervorgerufen durch *Nanophyes salmincola* Chapin. Arch. Scriffs- u. Tropen-Hyg. **30:** 396–399.

KNIGHT, R. A. AND PRATT, I. 1955 The life-histories of *Allassogonoporus vespertilionis* Macy and *Acanthatrium oregonense* Macy (Trematoda: Lecithodendriidae). J. Parasit. **41:** 248.

PERNOT, E. F. 1911 "Salmoning" of dogs. Oreg. Sta. Board of Health Bull. 5, pp. 1–21.

PHILIP, C. B., HADLOW, W. J. AND HUGHES, L. E. 1953 *Neorickettsia helmintheca*, a new rickettsia-like disease agent of dogs in western United States transmitted by a helminth. Internatl. Cong. Microbiol., Rept. Proc. 6th Cong., Rome **2:** 256–257.

———1954 Studies on salmon poisoning disease of canines. I. The rickettsial relationships and pathogenicity of *Neorickettsia helmintheca*. Exp. Parasit. **3:** 336–350.

PRICE, E. W. 1929 *Distomulum oregonensis* synonym of *Nanophyetus salmincola*. J. Parasit. **15:** 290.

SHAW, J. N. 1947 Some parasites of Oregon wild life. Oreg. Agr. Exp. Sta. Tech. Bull. No. 11, p. 8.

SIMMS, B. T. 1932 Pathogenicity of metacercariae of *Nanophyetus salmincola* Chapin for fish hosts. J. Parasit. **19:** 160.

———, DONHAM, C. R. AND SHAW, J. N. 1931 Salmon poisoning. Am. J. Hyg. **13:** 363–391.

——— AND MUTH, O. H. 1933 Salmon poisoning: Transmission and immunization studies. Proc. 5th Pac. Sci. Cong., Vancouver, B. C.

SINITSIN, D. F. 1930 Contribution to the life history of the salmon-poisoning fluke of dogs, *Nanophyetus salmincola* (Chapin). J. Parasit. **17:** 57–58.

SKRJABIN, K. J. AND PODJAPOLSKAJA, W. P. 1931 *Nanophyetus schikhobalowi*, n. sp. ein neuer Trematode aus dem Darm des Menschen. Zbl. Bakt. 1. Abt. Orig. **119:** 294–297.

SUCKLEY, GEO. 1855 Report of explorations and surveys to ascertain the most practicable and most economical route for a railroad from the Mississippi to the Pacific Ocean made under the directions of the Secretary of War, 1853–1855. Book 2, pt. 3, Zool. Rept., p. 112.

WALLACE, F. G. 1933 The life history of *Troglotrema mustelae* n. sp. J. Parasit. **19:** 164.

———1935 A morphological and biological study of the trematode, *Sellacotyle mustelae* n. sp. J. Parasit. **21:** 143–166.

WARD, H. B. AND MUELLER, J. F. 1926 A new pop-eye disease of trout-fry. Arch. Schiffs- u. Tropen-Hyg. **30:** 602–609.

WITENBERG, G. 1932 On the anatomy and systematic position of the causative agent of so-called salmon poisoning. J. Parasit. **18:** 258–263.

EXPLANATION OF PLATE I.

FIGURE 1. The adult fluke, *Nanophyetus salmincola* (Chapin).

FIGURE 2. Dorsal view of the reproductive systems of *N. salmincola*. Right and left vitelline ducts not extended to area of vitelline glands. Vitelline glands omitted.

FIGURE 3. Newly passed egg of *N. salmincola*.

FIGURE 4. Embryonated egg containing motile miracidium.

FIGURES 5 AND 6. Free-swimming miracidia.

FIGURE 7. Mature redia containing numerous cercariae and germ balls.

FIGURE 8. Immature redia containing only germ balls.

FIGURE 9. Microcercous xiphidocercaria of *N. salmincola*.

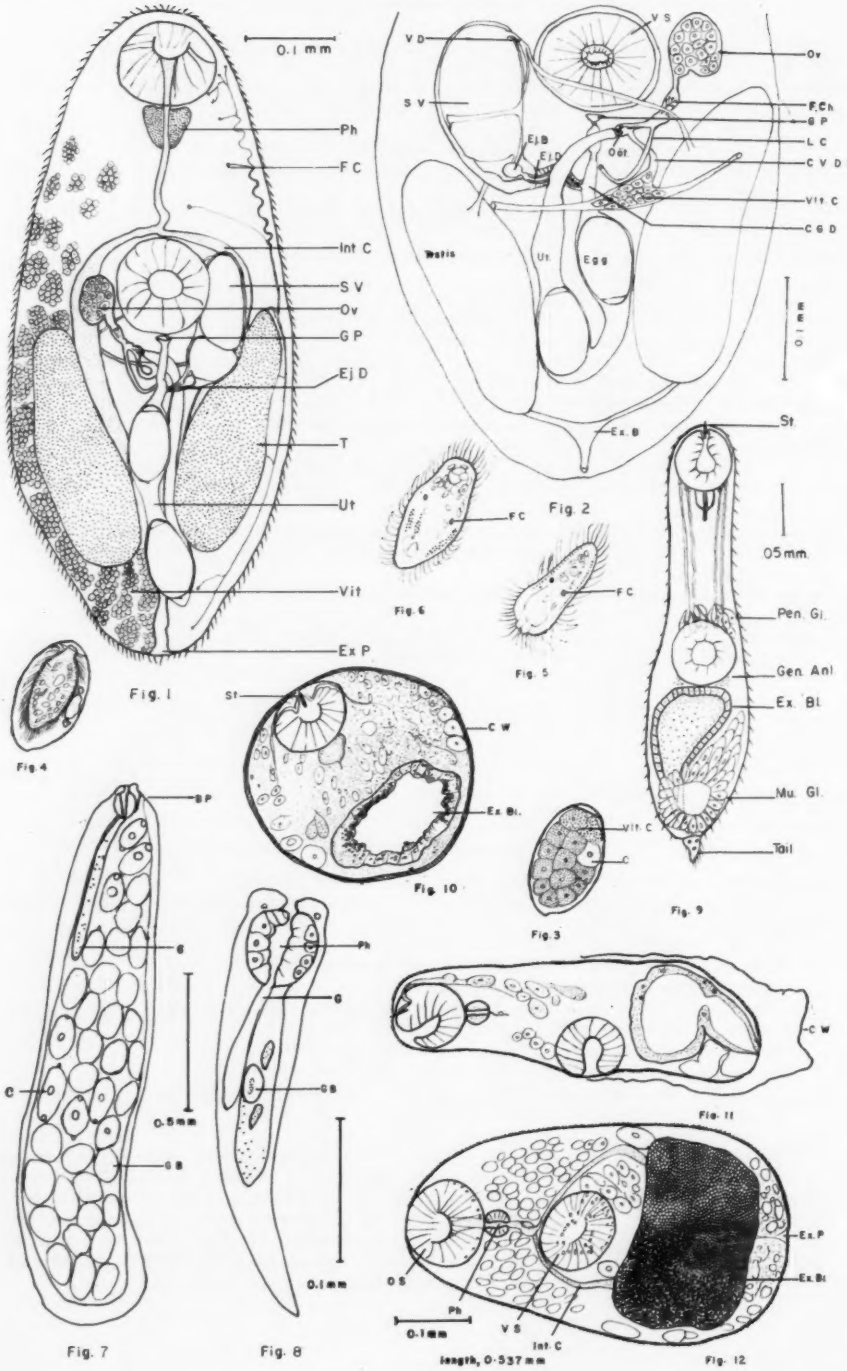
FIGURE 10. Developing metacercaria 2 days after encystment.

FIGURE 11. Developing metacercaria ruptured from 2-day-old cyst.

FIGURE 12. Infective metacercaria taken from a "wild" fish.

Abbreviations: BP, birth pore; C, cercaria; CGD, common genital duct; CVD, common vitelline duct; CW, cyst wall; EjB, ejaculatory bulb, EjD, ejaculatory duct; ExBl, excretory bladder; ExP, excretory pore; FC, flame cell; FCh, fertilization chamber; G, gut; GB, germ ball; GP, genital pore; Gen Anl, genital anlagen; Int C, intestinal cecum; LC, Laurer's canal; Mu Gl, mucous gland; Oot, ootype; O, ovum; OS, oral sucker; Ov, ovary; Pen Gl, penetration gland; Ph, pharynx; SV, seminal vesicle; St, stylet; T, testis; Ut, uterus; VD, vas deferens; Vit, vitellaria; Vit C, vitelline cells; VS, ventral sucker.

PLATE I


0.1 mm
Ph
F C
Int C
S V
Ov
G P
Ej D
T
Ut
Vit
Ex P
Fig. 1
V S
V D
Ov
S V
F.Ch
G P
Ej B
Ej D
L C
Oot.
C V D
Vit. C
C G D
Testis
Ut.
Egg
Ex. B
Fig. 2
F C
Fig. 6
F C
Fig. 5
St
05mm.
Pen. Gl.
Gen. Anl
Ex. Bl.
Mu. Gl.
Tail
Fig. 9
St
C W
Ex. Bl.
Fig. 10
Fig. 4
Vit. C
C
Fig. 3
G P
G
C
0.5mm
G B
Ph
G
G B
0.1mm
Fig. 7
Fig. 8
C W
Fig. 11
Ex. P
Ex. Bl.
O S
Ph
0.1mm
V S
Int. C
length, 0·537 mm
Fig. 12

THE SOUTH AFRICAN TICKS *RHIPICEPHALUS CAPENSIS* KOCH AND *R. GERTRUDAE* N. SP.*

B. FELDMAN-MUHSAM

Department of Parasitology, The Hebrew University, Jerusalem, Israel

The specific status of the Cape brown tick *Rhipicephalus capensis* Koch, 1844, is not quite clear. Some authors (Neumann, 1905, 1907; Dias, 1953) divided *R. capensis* into several subspecies which others subsequently synonymized (Zumpt, 1942, 1950; Theiler, 1943) or elevated to specific rank (Dias, 1951; Feldman-Muhsam, 1956).

While studying some south African rhipicephalids (Feldman-Muhsam, 1956) it was noticed that material determined by several acarologists as *R. capensis* was not homogeneous and showed marked variations in many characters. This concurs with Zumpt's (1942, p. 483) presumption that *R. capensis* is an extraordinarily variable species. It appears, in fact, that if certain individual characters are considered separately, specimens determined generally as *R. capensis* can be arranged in a continuous series of gradual variations. But if a complex of such variable characters is considered as a whole, the differentiation of types is feasible. Since these types are also found to be constant in one or more characters, particularly the genital aperture of the female, their elevation to specific rank seems to be justified. It may be noted that this state of affairs is quite common throughout the *Ixodidae*.

The examination of the mounted genital aperture of the female of specimens considered to be *R. capensis* showed that there are at least 2 distinct types with no transition forms. We thus came to the conclusion that *R. capensis* includes 2 distinct species. These species are readily diagnosed by several macroscopic characters in the males as well as in the females. One type of *R. capensis* is represented by medium-sized specimens, red-brown in color, with relatively long, wide, and expanding cervical grooves, generally quite distinct median and paramedian grooves, and with the so-called "characteristic" anal armature of the species (fig. 1,C). The second type of the so-called *"R. capensis"* is represented by smaller dark or light brown specimens, with short, deep, crescent-like cervical grooves, with less distinct median and paramedian grooves, and relatively small, rounded anal plates.

After examining Koch's type specimen from the Berlin Museum (kindly sent to us for examination by Dr. W. Crome), we came to the conclusion that the first type represents the true *R. capensis,* whereas the second represents a distinct species and should be given a new name. We propose to call the new species *R. gertrudae,* in honor of Dr. Gertrud Theiler, through whose courtesy most of our material was obtained.

MATERIAL EXAMINED

(1) Koch's holotype of *R. capensis*—1 male, from "Capland" (Berlin Museum).
(2) 1 female, "Capland" (Berlin Museum No. 1101, probably from Koch's collection). Determined by Zumpt as *R. capensis.* Is actually *R. gertrudae.*

Received for publication April 10, 1959.

* This investigation was supported by research grant R.G. 4531 from the National Institutes of Health, U.S. Public Health Service.

(3) Laboratory bred males and females of *R. capensis* (Breed No. 2826), bred by Dr. G. Theiler. These are *R. capensis* s.s. Mother tick from Gift Bergen, Cape Province, January 1, 1942.
(4) Males and females from Dr. Theiler's Namaqualand "Tick-Survey" and determined by her as *R. capensis*. The batch contains *R. gertrudae* (22 females and 7 males) and *R. capensis* s.s. (3 females and 10 males). (Information on host, date, and exact locality not supplied by collector.)
(5) 3 males and 3 females from Swellendam, 1902 (No. 2662 ii). Determined by Neumann as *R. capensis*. All are *R. gertrudae*.
(6) 8 females and 5 males from Clanwilliam (No. 2662 V) from Lounsbury Collection. All are *R. capensis* s.s.
(7) 1 male and 1 female (South Africa) *R. gertrudae*.
2 males and 1 female (South Africa) *R. capensis* s.s.

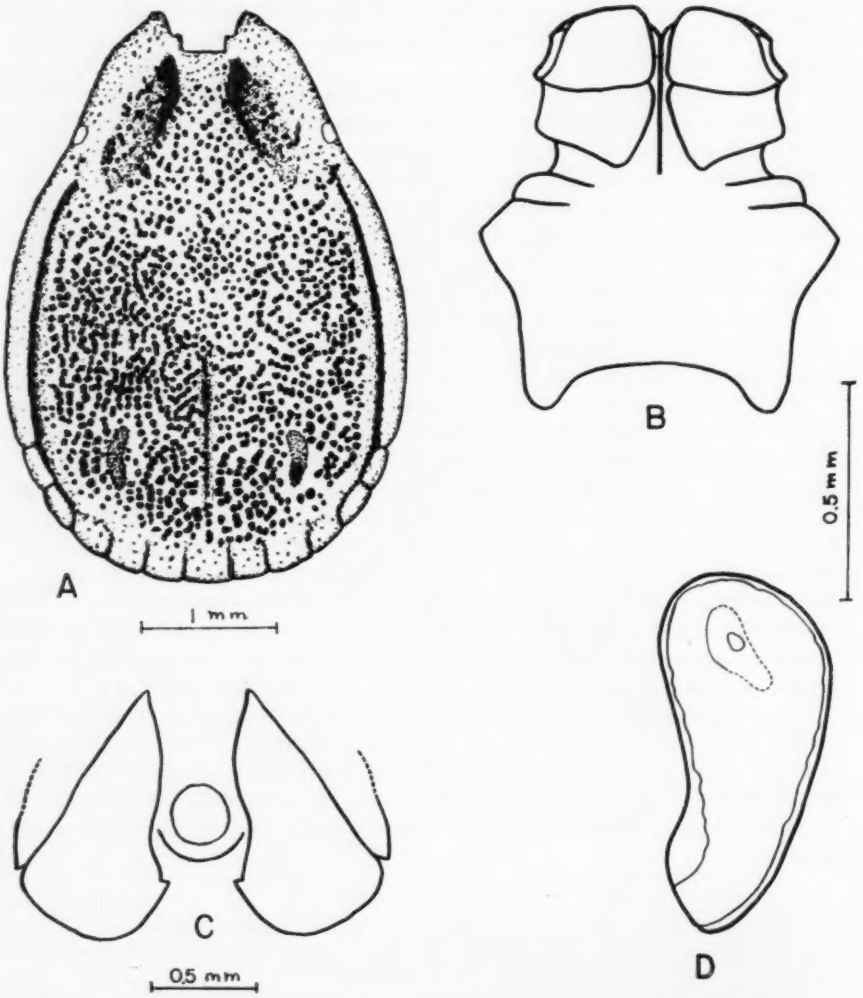


FIGURE 1. *Rhipicephalus capensis* K. Male holotype. A, scutum; B, capitulum; C, anal plates; D, stigma.

(8) A tube of males and females of mixed origin in South Africa. Determined by Dr. Theiler as *R. capensis.*
The batch contains *R. gertrudae* (16 females and 4 males) and *R. capensis* s.s. (6 females and 5 males).
(9) 4 males and 4 females (No. 2661 ii), 21.3.1900. Determined by Neumann as *R. capensis.*
1 male and 1 female are *R. capensis* s.s.
3 males and 3 females ? (Perhaps new species).

DESCRIPTION OF SPECIES

Rhipicephalus capensis Koch, 1844, s.s.

Male:

Description based on Koch's holotype.

Scutum 4 mm long and 2.8 mm wide, pear-shaped, red brown in color. Area between the marginal grooves uniformly and densely punctated with fairly deep and large dots which are partly confluent and display a shagreen appearance. Cervical grooves extending posteriorly beyond the eyes, deep in their anterior part but becoming gradually shallow laterally and posteriorly. Marginal grooves wide, deep and well defined, enclosing 2 festoons at their posterior part, and in the anterior part almost reaching the level of the eyes. Median and paramedian grooves shallow but well defined and quite wide (fig. 1,A). Eyes big and slightly bulging. First coxa visible from the dorsal side. Second and third coxae having 1 spine each, but the fourth with 2. Stigma comma-shaped and tail medium wide (fig. 1,D). (It is somewhat narrow in most specimens.) Anal plates big, the antero-lateral margin almost straight; postero-lateral angle a rounded-off right angle; median post-anal angle forming a small pointed denticle (fig. 1,C). Legs increasing in size progressively and considerably from pair I to IV. Cornua long (fig. 1,B).

Female:

(As the female was unknown to Koch and therefore no female type ever existed, our description is based on material bred in the laboratory by Dr. Gertrud Theiler (Breed No. 2826). One female of this breed, in our own collection, is suggested as female allotype; others have been sent to Dr. Theiler.)

Female of *R. capensis* s.s. 3.4 mm long and 2.2 mm wide. Color of the scutum, capitulum and legs red brown, alloscutum dark brown. Cervical grooves deep and long, moderately bent medially. Lateral grooves of scutum well defined, and with a lateral border. Area between lateral grooves densely and uniformly punctated with fairly large and deep, often confluent dots. Area external to the lateral grooves densely punctated with medium size dots (fig. 2, C).

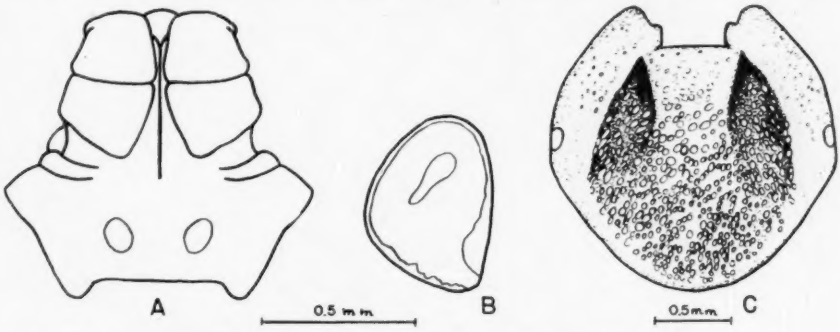


FIGURE 2. *Rhipicephalus capensis* K. Female. A, capitulum; B, stigma; C, scutum.

Scutum almost as long as wide (1.88 by 1.92 mm). Eyes slightly bulging. Cornua prominent (fig. 2, B). The entrance of the genital aperture into the vaginal tube V-shaped, with rounded bottom. Internal borders of the flaps convex medially (fig. 6). Mounted genital aperture of *R. capensis* s.s. resembling very much that of *R. compositus* (syn. *R. ayrei* Lewis, 1933), but the "cup" of the genital aperture of *R. capensis* s.s. much less pigmented than that of the cotype of *R. ayrei* (from the British Museum) or those of the laboratory-bred specimens of Miss J. B. Walker kindly placed at our disposal for examination (Feldman-Muhsam 1956, plate I, fig. 2).

R. gertrudae n. sp.

Male:

(The holotype male has been selected from a large batch of ticks collected in Namaqualand and determined by Dr. G. Theiler to be *R. capensis.* This specimen is kept in our collection. Paratypes from the same batch have been sent to Dr. Theiler.)

Scutum 2.6 mm long and 1.9 mm wide, and red brown in color, densely and uniformly punctated (less dense than in *R. capensis* s.s.) with fairly large dots, not confluent. Cervical grooves deep, very short, and crescent-like. Marginal grooves narrow and well defined in the type specimen, but more superficial in certain other specimens, anteriorly not approaching the eyes as closely as in *R. capensis* s.s., posteriorly generally including only 1 festoon; formed by juxtaposed large dots. Median and paramedian grooves small, narrow, and superficial, in some specimens hardly perceptible (fig. 3,A). Stigma very wide relative to its length and, consequently, the tail seeming short (fig. 3,D). The anal plates smaller than in *R. capensis* s.s. and much more rounded off in all their angles as well as at the antero-lateral margin (fig. 3,C). Size of the legs not increasing as considerably from pair I to IV as in *R. capensis* s.s.

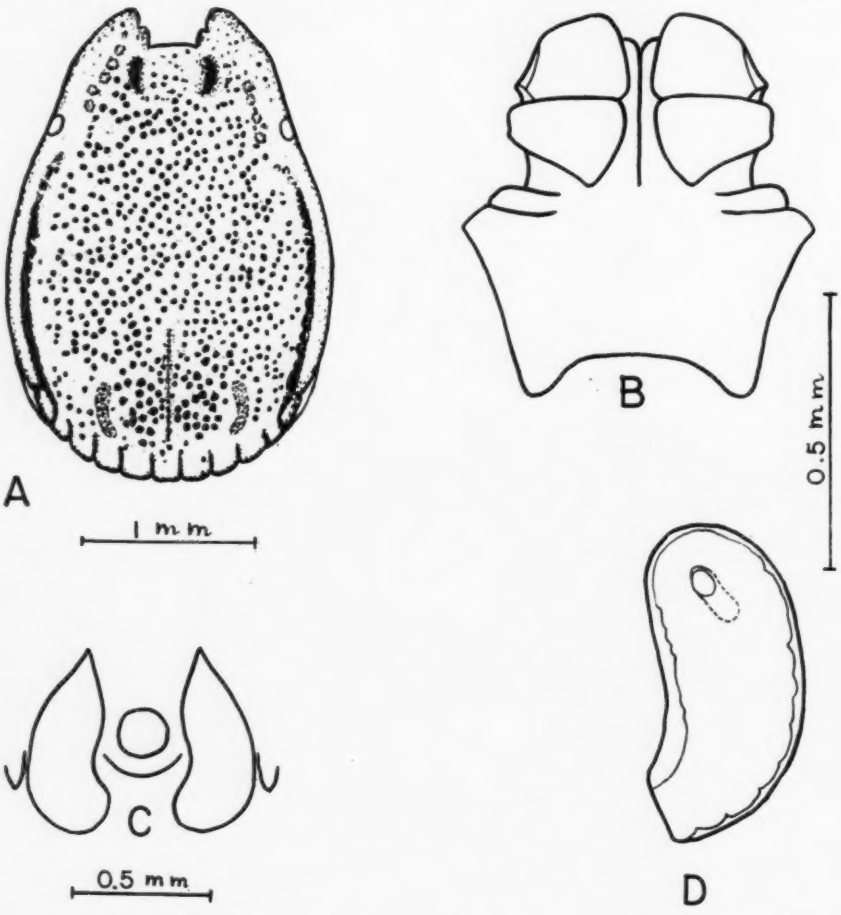


FIGURE 3. *Rhipicephalus gertrudae* n. sp. Male type. A, scutum; B, capitulum; C, anal plates; D, stigma.

Female:

(The allotype female is from the same batch as the male and is kept in our collection. Paratypes have been sent to Dr. G. Theiler.)

Female of *R. gertrudae* smaller than *R. capensis* s.s. Color of the type red-brown, but specimens examined sometimes dark red-brown and others light yellow-brown. Scutum slightly wider than long (1.62 by 1.5 mm), uniformly punctated with fairly large dots (sparser than in *R. capensis* s.s.), but the scapulae and the area near the eyes almost smooth with only a few large and some fine dots (fig. 4,C). Cervical grooves, as in the male, crescent-like at their

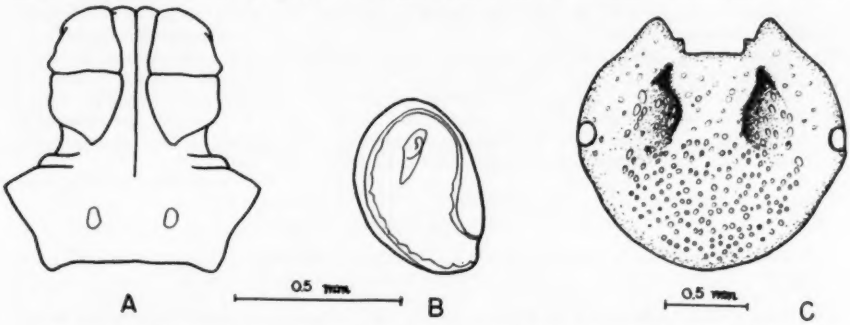


FIGURE 4. *Rhipicephalus gertrudae* n. sp. Female type. A, capitulum; B, stigma; C, scutum.

anterior end, and becoming convex medially, generally shorter than in *R. capensis* s.s. Lateral grooves merely indicated or may be absent. Cornua short (fig. 4,A). Mounted genital aperture very characteristic. The "cup" small, about two-thirds the size in *R. capensis* s.s.; its bottom flat or almost so, and the anterior portion of the flaps bent laterally (fig. 5). (This character is

FIGURE 5. *Rhipicephalus gertrudae* n. sp. Female genital aperture. ×150.
FIGURE 6. *Rhipicephalus capensis* K. Female genital aperture. ×150.

so far the easiest and most reliable differential diagnostic character for the female of this species. However the unmounted genital aperture is not suitable as a differential character for this species.)

Key to Males of "Capensis" Group

1. Big, subtriangular anal plates, with antero-lateral margin generally straight, but with rounded angles 2
   Lateral and posterior margin of anal plates uniformly curved 3
2. Scutum with large and deep punctations, well separated from each other. Area external to the marginal grooves almost smooth, but with fine and inconspicuous punctations. Large ticks, about 4.5 mm long. ..... *R. compositus* Neumann, 1897
   Punctations not as large, but partly confluent. Scutum having a shagreen appearance. Area external to the marginal grooves less smooth, punctated with many small dots. Medium-sized ticks, about 3.5 mm long. ..... *R. capensis* (Koch, 1844) s.s.
3. Scutum convex, with fairly large punctations evenly distributed (not as dense as in the above species). Cervical grooves very short and crescent-like. Marginal grooves formed by juxtaposed large dots, closing one festoon. Anal plates inverted comma-shaped. Small ticks, 2.5 to 3 mm long. ..... *R. gertrudae* n.sp.
   Scutum flat, with punctations more dense. Marginal grooves wide and deep, closing 2 festoons. Anal plates sickle-like. Medium-sized ticks, about 4 mm long.
   R. longus Neumann, 1907

Key to Females of "Capensis" Group

1. Scutum between lateral grooves densely and uniformly punctated with large and deep punctations. Area external to the lateral grooves sparingly punctated with fine dots 2
   Difference in punctation between center of scutum and external area not so conspicuous ... 3
2. Lateral groove forming a distinct lateral border. Area external to lateral grooves and behind the eyes smooth and shiny and with hardly visible dots. Large ticks about 4.5 mm long. "Cup" of the mounted genital aperture as wide as deep. Flaps thick and strongly pigmented. Midparts of the internal borders of the flaps convex medially. *R. compositus* Neumann, 1897
   Lateral groove not as sharp as above and punctations between lateral grooves slightly smaller. Medium-sized ticks, about 4 mm long. "Cup" of the mounted genital aperture much deeper than wide. Entrance into vaginal tube V-shaped. Flaps long and thin, and with medially protruding projection. ..... *R. longus* Neumann, 1907
3. Lateral grooves well defined. Punctations partly confluent. Cornua long. Medium-sized ticks 3.5 to 4 mm long. "Cup" of genital aperture similar to *R. compositus*, but less pigmented. ..... *R. capensis* (Koch, 1844) s.s.
   Lateral grooves poorly defined or wanting, cervical grooves strongly bent medially. Punctations less dense. Cornua short. Small ticks about 2.5 mm long. "Cup" of genital aperture about two-thirds size for *R. capensis* s.s. Bottom of the opening of "cup" flat, and anterior portion of flaps turned laterally ..... *R. gertrudae* n. sp.

DISCUSSION AND CONCLUSIONS

The recognition of *R. capensis* s.s. and *R. gertrudae* as distinct species and the possibility of differentiating between them and other related species enabled us to clarify some obscurities in the literature on the classification of the "capensis" group of ticks.

It is true that *R. capensis* s.s., *R. gertrudae, R. compositus* and *R. longus* resemble each other in many respects. For instance the anal armatures of the males of *R. capensis* s.s. and of *R. compositus* are very similar. The punctation of the scutum of the males of *R. compositus* resembles that of *R. longus*. The scuta of the females of *R. capensis* s.s., and *R. longus* are very similar (Zumpt says that they cannot be distinguished). The mounted genital aperture of the female of *R. capensis* s.s. resembles that of *R. compositus*.

Under these circumstances it is not surprising that Zumpt, who examined extensive material and was well acquainted with the different patterns occuring in that group, failed to recognize that they represent different and distinct species. In 1942 and 1950 he accepted only one species and one subspecies, viz., *R. capensis* (sensu Zumpt) and *R. capensis longus*. His 1942 drawings of the male (figs. 1, 2)

were those of *R. capensis* s.s., whereas that of the anal plates (fig. 4) depicted *R. gertrudae*. His drawings of the female (fig. 9) was also characteristic of *R. capensis* s.s. In his introductory note to the capensis group he says that "specimens of *capensis* with finer and sparser punctation as well as with more or less rounded off, respectively reduced anal plates . . . are often diagnosed as *capensis* var. *compositus*". Zumpt considers that such specimens should be included in *capensis* (sensu Zumpt). In the light of our criteria these specimens are typical *R. gertrudae*.

Zumpt (1942) also considers, erroneously, Neumann's male type specimen of *R. compositus* to be a "surprisingly" large specimen of *R. capensis* (sensu Zumpt).

In Santos-Dias' paper on the ticks of Nyasaland (1951) the drawing of the male of *R. capensis* (fig. 3) is that of *R. capensis* s.s., while that of the female (fig. 4) is characteristic of *R. gertrudae*. (Hence it appears that *R. capensis* s.s. as well as *R. gertrudae* are present in Nyasaland.)

The present author's previous (1956) description of the female genital aperture of *R. capensis* really applies to *R. gertrudae*.

There is also much confusion in studies on the ecology and the geographical distribution of the capensis group. Theiler (1950), in her survey of the distribution of *R. capensis* in South Africa, says: "In *R. capensis* it is almost impossible from the data collected to get a clear picture of the role played by humidity and/or aridity", and further on: ". . . the data are still so incomplete and apparently so contradictory, that it is impossible at this stage to draw any conclusions as to the factors limiting the activity of the adult tick".

It is most probable that the reason for the contradictory results is that the data of two distinct species were pooled.

A comparison of the biology and taxonomy of pre-imaginal stages of laboratory bred material is most desirable and would throw further light on the relationship between *R. capensis* s.s., *R. gertrudae, R. compositus* and *R. longus*. Material diagnosed as *R. capensis* should be re-examined critically in order to correlate taxonomic studies with geographical distribution and the problem of disease transmission.

SUMMARY

South African ticks generally diagnosed as *R. capensis* Koch, 1844, are separated into two distinct species: *R. capensis* s.s. and *R. gertrudae* n. sp. Differential characters for the males and females of these species are described.

Diagnostic keys to the males and females of *R. capensis* s.s., *R. gertrudae, R. compositus* and *R. longus* are given.

The division of *R. capensis* s.l. into two species is likely to elucidate contradictory ecological observations.

REFERENCES CITED

DIAS, J. A. T. S. 1951 Subsídios para o estudo da fauna Ixodológica da província do Niassa. An. Inst. Med. Trop. **8:** 563–614.

——— 1953 Sobre uma nova subespécie de *Rhipicephalus* do "grupo *Capensis*" Zumpt: *R. capensis pseudolongus* n. ssp. Mem.e Est. Mus. Zool. Univ. Coimbra. No. 214.

FELDMAN-MUHSAM, B. 1956 The value of the female genital aperture and the peristigmal hairs for specific diagnosis in the genus *Rhipicephalus*. Bull. Res. Council Israel **5B:** 300–306.

NEUMANN, L. G. 1905 Notes sur les Ixodidés. Arch. Parasit. **9:** 225–241.

——— 1907 Description of two new species of African ticks. Ann. Trop. Med. Parasit. **1:** 115–120.

THEILER, G. 1943 Notes on the ticks off domestic stock from Portuguese East-Africa. Moçambique (33): 51–120.

——— 1950 Zoological Survey of the Union of South Africa. Tick Survey, Part IV. Onderstepoort J. Vet. Sci. & An. Ind. **24**: 7–22.

ZUMPT, F. 1942 Zur Kenntnis Afrikanischer Rhipicephalusarten. V. Vorstudie zu einer Revision der Gattung *Rhipicephalus*. Zeitschr. Parasitenk. **12**: 479–500.

——— 1950 Preliminary study to a revision of the genus *Rhipicephalus* Koch. Documentario "Moçambique" No. 60, pp. 57–123.

RESEARCH NOTE

THE EFFECT OF STILBESTROL IMPLANTS UPON MULTIPLE INFECTIONS OF *TRICHOSTRONGYLUS AXEI* AND *TRICHOSTRONGYLUS COLUBRIFORMIS* IN THE LABORATORY RABBIT.

Earlier experiments (Rohrbacher, 1958, J. Parasit. **44**: (supp.): 32) indicated that 12 mg stilbestrol implants had no effect upon the numbers of *Trichostrongylus axei* and *Trichostrongylus colubriformis* recoverable from grade California rabbits given a single infective dose of both species of nematodes.

As stilbestrol implants are widely used in the livestock industry, it seemed desirable to determine the effect of this hormone upon parasitized animals which would be exposed to more infective larvae following stilbestrol implantation. Therefore, the following experiment was designed in order to approximate more closely the natural conditions of multiple infection and to simulate more closely the natural usage of stilbestrol.

Forty-three grade California rabbits, raised free of helminth parasites on wire, were divided into a stilbestrol group and a control group (table 1). The animals ranged in age from 2 to 4 months, but all were of the same age within each of 4 replications. In 3 replications, both sexes were represented; in the 4th, only males were used. All animals were maintained on a standard pelleted rabbit ration, water, and salt ad lib. The nematodes used were obtained from pure infections of each species maintained in calves. Each rabbit in both groups received a total of 6,000 *T. axei* and 6,000 *T. colubriformis* given in doses of 1,000 infective larvae of each species on the 1st, 3d, 15th, 17th, 29th, and 31st day of the experiment. On the 24th day of the experiment, rabbits in the stilbestrol group were each given a 12 mg stilbestrol implant subcutaneously in the cervical region. Thirty-one or 32 days after the last infecting dose of larvae, all animals were killed and examined as described by Rohrbacher et al (1958, Am. J. Vet. Res. **19**: 625–631).

The parasite data are summarized in Table 1.

TABLE I. *Numbers of* Trichostrongylus *spp. recovered at necropsy.*

Group	Number of rabbits	Number of replications	Average number recovered	
			T. axei	*T. colubriformis*
Stilbestrol				
Males	13	4	905	1,277
Females	9	3	1,219	1,314
Control				
Males	12	4	858	815
Females	9	3	1,109	1,493

When the data were compared using Student's "t" test, no statistical difference could be found between the numbers of worms of either or both species of nematodes recovered from either the stilbestrol group or the control group or from either sex in each group. Therefore, under the conditions of this experiment, 12 mg stilbestrol implants had no effect upon infections with *T. axei and T. colubriformis* in the grade California rabbit.—GEORGE H. ROHRBACHER, JR., *Regional Animal Disease Research Laboratory, Agricultural Research Service, U. S. Dept. of Agriculture, Auburn, Alabama.*

A CAGE FOR MAINTAINING STOCK COLONIES OF PARASITIC MITES AND THEIR HOSTS*

JOSEPH H. CAMIN AND PAUL R. EHRLICH**
Department of Entomology, University of Kansas

A major problem in maintaining colonies of parasitic mites on their vertebrate hosts is that of cleaning the cage without unduly disturbing and destroying the parasites. With most cages now in use, either the excrement of the host must be allowed to accumulate indefinitely or a large proportion of the parasite colony must be destroyed each time the cage is cleaned. Often the colonies must be started anew after each cleaning. The investigator is faced with a choice of living with the odors of host animal wastes in order to maintain a continuously high level in his mite populations or he must gear his research to periodic fluctuations in the parasite populations and spend much time in routine maintenance. In our laboratory a cage has been designed that facilitates the routine removal of host waste products without disturbing the vast majority of the mites.

This cage (fig. 1) is essentially a cage within a cage. The outer container is a modification of the bird mite cage described by Chamberlain and Sikes (1950). It is a galvanized metal can with a moat soldered or welded around its upper edge. The circular form is less expensive to make than the rectangular form used by Chamberlain and Sikes. The can is 11 inches in diameter and 8 inches high. The moat is attached 2 inches from the upper edge of the can at an angle of 30°. It projects 1 inch higher than the can, and the outer diameter of the moat is 15 inches. A lid is fitted over the outer edges of the moat. The lid has a handle, 2 rectangular screened openings for ventilation, and a small hole through which the tube of a watering bottle may be inserted. With the lid in place, the water-filled moat serves the dual function of preventing the escape of mites and maintaining a high humidity within the can. Because the edge of the moat is an inch higher than the edge of the can, mites are unable to cross from the inner edge of the can to the lid. The watering tube, however, does provide such a bridge and must be lightly greased with petroleum jelly in order to close this avenue of escape.

The inner cage is a triangular, metal-framed insert, 9 inches on a side, with hardware-cloth walls (fig. 2). It is open at the top and bottom. A triangular lid of similar construction (a) is fitted over the top of the inner cage to prevent the host animal from reaching the outer lid and thus permitting the escape of mites. The inner cage lid is held in place by hooks (b) on 2 of the 3 sides. A triangular pan (c) fits within the inner cage and serves as a receptacle for host feces and urine. The floor (d) of the inner cage is constructed of hardware cloth and allows host excrement to drop through into the pan. The floor rests on metal stops (e) that are welded onto the inner walls of the pan. The sides of the pan are slightly crimped (f) so that lifting the floor also lifts the pan.

Received for publication September 25, 1959.
* Supported in part by Grant No. E-2487(C1) from the National Institute of Allergy and Infectious Diseases. Contribution number 1060 of the Department of Entomology, University of Kansas.
** Present address: Natural History Museum, Stanford University, California.

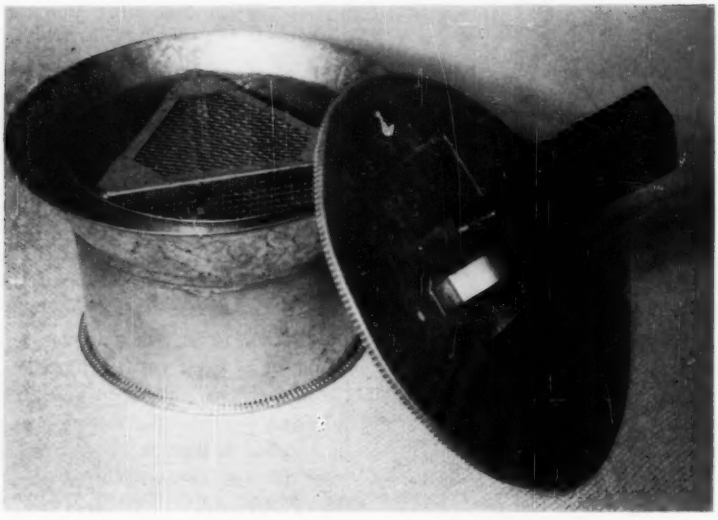


FIGURE 1. Culture cage assembled, showing moat on outer cage, triangular inner cage within the outer cage, and the three mite-nesting spaces between the walls of the inner and outer cages.

When the triangular inner cage is placed within the circular outer cage, 3 large spaces are left between the walls of the two components (fig. 1). These spaces are filled with shredded paper, corrugated cardboard, wood shavings, or other suitable nesting materials to within an inch of the top of the cage. The host animal

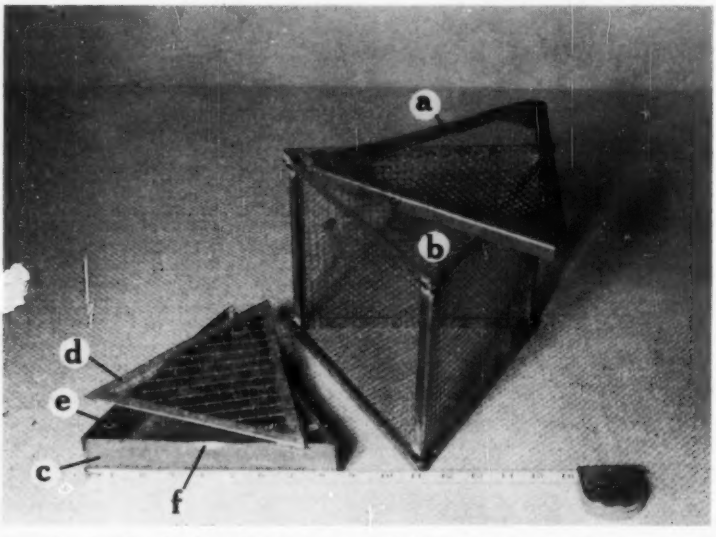


FIGURE 2. Details of triangular inner cage. (a) inner cage lid, (b) hook for securing lid to cage, (c) pan for collecting host waste products, (d) floor of inner cage, (e) stop or bracket upon which the floor rests, (f) crimped edge of pan.

is placed in the triangular inner cage. The nidicolous ectoparasitic mites, such as the chicken mite (*Dermanyssus gallinae*), the spiny rat mite (*Echinolaelaps echidninus*), and the snake mite (*Ophionyssus natricis*), all of which are being reared in our laboratory, spend most of their time in the nesting material and come to the host only to feed. At regular intervals when host excrement accumulates in the triangular pan, the host is temporarily removed from the cage, and then the pan and the floor of the inner cage are lifted through the triangular cage. The pan is emptied and the pan and floor are washed and placed back into the cage. A few mites are lost during this procedure, but because the triangular cage and the nesting material between the inner and outer cages are left in place, the vast majority of the mites are undisturbed. Thus, sanitary conditions can be maintained without disrupting the colony of parasites.

LITERATURE CITED

CHAMBERLAIN, R. W. AND R. K. SIKES 1950 Laboratory rearing methods for three common species of bird mties. J. Parasit. **36:** 461–465.

RESEARCH NOTE

HUMAN AND ANIMAL SCHISTOSOMIASIS IN KHARTOUM PROVINCE, SUDAN.

Early records refer to very low prevalence of urinary schistosomiasis and the complete absence of the intestinal type due to *Schistosoma mansoni* in Khartoum Province. Agricultural pump schemes have apparently aggravated the situation, and schistosomiasis haematobia is actually much more prevalent than was believed. The highest reported infection rate recorded was among children in certain agricultural schemes (Annual Report Ministry of Health, Sudan Government, 1953–54), of which 10.86 percent were found infected. In an agricultural community in Shambat (6 miles north of Khartoum), not surveyed by the health authorities, the writer found, with only one urine examination (sedimentation technique), that out of the 439 schoolboys 6 to 12 years old, 102 or 23.2 percent were infected with *S. haematobium*. Most of these boys had pronounced hematuria. Only 3 girls out of 395 of about the same age as the boys were found infected in the 2 village girls' schools; 19 had only red blood corpuscles in their urine. The low infection rate among the girls is due to social customs.

All cases are autochthonous. The snail intermediate host, *Bulinus truncatus*, and the potential host, *Bulinus forskalii*, occur in large numbers in irrigation canals in the area. Infected *B. truncatus* were encountered (Malek, Proc. 6th Intern. Cong. Trop. Med., Lisbon, in press). The infection rates among these snails varied seasonally. Usually the prevalence of schistosomiasis is low where there is lift irrigation, since fluctuation in water level hampers the establishment of large snail colonies. However, the canals at the Shambat scheme contain many deep parts which, together with the ends of the canals, harbor large snail populations.

Neither *S. mansoni* nor its intermediate hosts have been recorded in this area. The writer is recording here for the first time *Biomphalaria rüppellii* in Khartoum Province. The snail is found in one canal in the Abu el-Ela agricultural scheme, 9 miles north of Shambat scheme. The snail is abundant, together with *Bulinus truncatus* and *B. forskalii*, toward the dead end of a 2.5-mile-long canal. Shorter canals in the same scheme harbor *Bulinus* but not *Biomphalaria*. Examination of stools of 8 natives who frequent the canal harboring *Biomphalaria* were negative for *S. mansoni*. Nevertheless, the presence of *Biomphalaria* in this locality, and possibly in other localities in the province, presents a threat of the disease being introduced and spread.

Schistosomiasis due to *S. bovis* is also present in the Province, though the prevalence is much lower than that of *S. haematobium*. It has been encountered in cattle, sheep, pigs, equines, and camels. In certain cases, however, the writer could not be sure whether these animals became infected in Khartoum Province or elsewhere. The worms varied in size, being largest in the pig and sheep. There was a predominance of male worms over females more obvious than in infections with other species of schistosomes. Examination of small mammals for their natural infection with schistosomes is being carried out. Of those examined so far, 3 mongooses, 6 Nile rats, and 4 gerbils were negative.—EMILE ABDEL MALEK, *University of Khartoum, Sudan.* (Present address: *Tulane University Medical School, New Orleans.*)

SOCIETY BUSINESS

THE SOCIETY SEAL.

The recent AIBS meetings, at Pennsylvania State University, were marked by the adoption of an official seal by the American Society of Parasitologists. As designer of the seal, I have been asked to prepare a short statement on its origin and interpretation.

Without going into details, which are a matter of record with the secretary, it may be stated that the present design was executed only after months of deliberation with various officers and members of the council. This exchange made it clear that the weight of opinion was in favor of a modern treatment with incisive simplicity. It was my own conviction that the seal must be a good design in itself, apart from any meaning it may convey, so that only harmonious elements could be admitted. Within this limitation however, it was felt that the seal should be as rich in symbolism as possible. The result is now on view.

Although the first meetings of the society were not held until 1925, the actual date of organization was December 1924, and hence that date appears on the seal. Two variants of the seal have been worked up: one with a simple line treatment throughout, the other with the lettering appearing on a black border. Each will be useful for certain purposes. Both are official.

The main field of the design consists of a cross section of a nematode esophagus. A nematode is a fitting basic motif for the seal because of the tremendous impetus given to parasitology by the early hookworm campaigns. The nematode esophagus is furthermore a classical example of eutely, cell constancy, and thus symbolizes the contributions of parasitologists to cytology.

This design is superimposed upon a flatworm sucker, such as occurs in most orders of trematodes and cestodes. Thus the three main groups of helminths are represented, and by extension may stand for such others as acanthocephans and leeches. The short heavy parallel lines at the corners of the lumen are not just decorations, but are the *Kanten Fasern*, skeletal elements present in the esophagus of many nematodes. In the angles of the "Y" there appear an *E. histolytica* cyst for protozoology, a planorbid snail, representing not only snails as trematode vectors, but the concern of parasitologists with invertebrates in general, while the anopheline egg at the top represents medical entomology. The anopheline egg was chosen because it is the one insect egg readily identifiable from a simple outline. Various other arthropod shapes were tried, but refused to fit in with the requirements of the composition. It is felt that the mosquito merits a place on the seal because of the importance of mosquitoes as pests in their own right, and as vectors of viral, protozoal, and worm diseases. Moreover, the mosquito has a certain romantic aura which is not shared by most lethal insects.

Looking at the design another way, the central motif represents a sunburst, while alternately appearing between the rays is a marginal row of small circles denoting rain drops. Rain and sun are the two principal climatic determinants in the ecology and distribution of parasites and their hosts. If one ignores for a moment the egg, snail, and cyst, the white areas and central circle remaining form the symbol for the most up-to-date tool of biochemical and physiological investigation: the radioisotope.

Thus the seal includes not only the historic interests of parasitologists: morphology, taxonomy, life histories, and cytology; but also represents the more modern concern with questions of zoogeography, ecology and distribution, biochemistry, and parasite physiology.

Three elements of the seal: sun, rain, and mosquito appear (although in different form) on the seal of the American Society of Tropical Medicine and Hygiene. It is felt that this is altogether appropriate for two societies which periodically meet together, and whose interests and membership to such a large extent overlap.—JUSTUS F. MUELLER.

THE AWARD OF THE FIRST HENRY BALDWIN WARD MEDAL

Mr. President, Members and Guests of the Society:

It is a great privilege to act for the Society in presenting its first award of the Henry Baldwin Ward Medal.

This award was made possible through the generosity of Parke, Davis and Company, and consists of a bronze medal, $1,000, and provision for travel to the meeting. It is highly appropriate that it should be named in honor of Henry Baldwin Ward. He was among the earliest American contributors to the literature on parasites, was the first to establish a graduate training center for parasitologists in this country, and was for many years a dominant figure in the Society's affairs; he founded the Journal of Parasitology and was the Society's first President. Accounts of his contributions and of his good influences on the history of the Society were recorded on the occasion of his gift of the Journal of Parasitology to the Society in 1932, and again following his death in 1945 (J. Parasit. **19:** 99; **32:** 323).

DR. CLARK P. READ

As first recipient of the Medal, which by its name implies an expression of high appraisal of his works, your Comfittee on Awards has selected Dr. Clark P. Read. I am sure that all of you as members of the Society experience the same great pride as does your Committee in recognizing in this manner one of our younger colleagues who, almost within the first decade of his professional career, has gained a firm place in the succession of leadership in the Society and in the field of parasitology. His first two papers, one a report of *Spirometra* in Texas cats, the other a description of a new nematode parasite of the spiny dogfish, were published in the Journal of Parasitology in 1948, eleven years ago.

Dr. Read was born in Texas in 1921. He did his undergraduate and graduate studies in that state at the Rice Institute under Professor Asa C. Chandler who, before his death last year, had invited Dr. Read to become joint author of his well-known textbook of parasitology. Upon receiving his Ph.D. in 1950, Dr. Read became Instructor and later Assistant Professor of Zoology at University of California in Los Angeles. After four years, he moved on to the

Johns Hopkins School of Hygiene to continue the high level of teaching, graduate student training, and research in parasitology maintained there for many years. A few months ago, announcement was made of his return to the Rice Institute as Professor of Parasitology.

Dr. Read's scientific works are many, more than thirty, and they represent to a remarkable degree the successive historical developments in parasitological research. After several early years of skillful work on the morphology and taxonomy of cestodes, trematodes, and nematodes, he began working on the physiology of helminths and protozoa, and by use of biochemical and other technics essentially unknown to his early predecessors, he has in recent years made numerous advances in the knowledge of parasites and parasitism. He is today a recognized authority on basic host-parasite relationships and has been a participant in several symposia on that subject. Along with this active program of teaching and research, Dr. Read has for some time served the Society as a member of the editorial board of its journal.

It is, therefore, with great pleasure that for the American Society of Parasitologists I present to you, Dr. Read, the first Henry Baldwin Ward Medal.

(*Presentation by* DR. PAUL C. BEAVER, *Chairman of Awards Committee.*)

THE ACCEPTANCE OF THE HENRY BALDWIN WARD MEDAL

My deep appreciation is expressed to the Awards Committee and to the Society for the great honor accorded to me in the awarding of this medal. I must also express gratitude to Parke, Davis and Co. which so generously endowed the award allowing the medal to be accompanied by a pleasing number of units of U. S. currency.

It is trite to say that no man is an island but this seems to be particularly true in my own case. A great many persons have aided, abetted, and influenced my professional career. Some of these have been ahead of me in professional experience, some behind. I really cannot say that one has been more important than another since I owe them thanks for different reasons. I should like to mention some of these people and indicate the twists which each has lent to my professional development. Since I, unfortunately perhaps, cannot divorce my personal life from my professional, some of my annotations are tributes with a strong emoitional basis.

I shall first mention Professor E. S. Hathaway, at that time Professor of Zoology at Tulane University, who introduced me to scientific research as an undergraduate. Although not a highly productive research man, Professor Hathaway was an exacting taskmaster, an unmerciful critic, and, most important, one who could share the excitement of investigation, even if the experiment failed to support the hypothesis.

It is appropriate to mention Dean Chauncey Leake, formerly of the University of Texas School of Medicine, who, when I decided that medicine was not an appropriate career, abetted my leaving medical school. He stood alone in this regard, since my classmates and several members of the medical faculty suggested that such a decision must be a symptom of a basic disorder of the central nervous system. Dr. Leake may still wonder why he received a Christmas card in 1956 from someone whom he had undoubtedly forgotten with the message "We were right ten years ago."

It would be redundant for me to eulogize Dr. Asa C. Chandler. This has been done on several occasions since his death a year ago. However, I can mention some special sorts of opportunity I received from Chandler. First, he loaded me with responsibility, but always slightly short of what I could handle. He apparently recognized that I seem to thrive on pressure. Hence, because I came to graduate study without a Bachelor's degree, he insisted that I submit two theses for the Master's degree in 1948. One of these was a study of the nematode genus *Capillaria,* published in 1949, and the other a trematode life history which is still unpublished. During the early portion of this same spring of 1948, Chandler suffered a severe heart attack and handed me the assignment of teaching his courses for most of a semester. During the summer of 1948 Chandler sent me to Oak Ridge, Tennessee, for a summer course and in the fall of that year I carried out a large part of the teaching in a new course in Radiobiology at the Rice Institute. My point is that Chandler taught me that I could usually do more than seemed practical at first glance. I learned many other lessons from this man since he was my major teacher throughout graduate study.

The fourth man I wish to mention has had a profound effect on me, an effect of which he is probably unaware. It gives me a great deal of pleasure to make him aware of it in the presence of this group. This man is Professor Gordon Ball of U.C.L.A. In my eyes Dr. Ball is a real giant of personal and scientific integrity. When I joined the faculty at U.C.L.A. as an instructor in 1950, Dr. Ball was Professor of Zoology. A lesser man than Gordon Ball would have expected a novice to assume a junior role in the scheme of things. However, Gordon will give you as much rope as you want. When you become entangled he will cut the rope and hand it back to you. We shared many experiences in teaching and departmental duties and I profited

immeasurably from the intangible assets of Professor Ball's personality.

I must express my appreciation to Dr. Frederik Bang of Johns Hopkins University who has lent great impetus to my career of the past five years. Intellectual stimulation and opportunities for broad, free-wheeling discussion and argument were fostered by Dr. Bang.

My deepest feelings involve my present and former graduate students. There are 16 of these men and I shan't name them. Each of them knows who he is. Graduate students mean a great deal to me. We have shared research experience, harebrained ideas, critical examination of the work of others, and such sub-professional activities as a glass of beer. I have learned something from each of them. I am proud of the fact that I have co-authored a number of publications with these men. Parenthetically, I should like to say that in past years I have never co-authored a paper unless I personally did a substantial portion of the laboratory work and in most cases prepared the paper for publication.

Last, but not at all least, I must publicly express my gratitude to my wife, Lee, who has borne my children, typed my manuscripts, and regulated the valve on my periodically inflated and deflated ego.

I have had some deep doubts concerning the appropriateness of the choice of recipient for this award but I will say that I will do my best to earn your esteem in the future. These doubts of mine have been particularly sharp in recent months since I have been asked twice in this period to review my research accomplishments. I found such a review to be a startling experience. My accomplishments taken in some sort of order appeared to be rather incomplete and, to be honest, rather insignificant. I strongly recommend such a review to all men between the ages of 30 to 40 when pride goeth before middle age. In my own case it seems to have had a salutary effect in enabling me to conclude that my most productive years must lie ahead, the rationalization being that surely these behind are not the most productive.

Therefore, rather than spending more of your time reviewing my past I should like to speak to the young man who will receive this award in 1969. Perhaps you completed your graduate work in June or will complete it in a year or two. You are indeed fortunate. You are beginning your career in times when parasitology offers the greatest variety of research excitement and fulfillment. You came into the world at a time when high rates of technological development allow new attacks on old problems and formulation of new problems. With new tools you can rephrase the questions asked of Nature. For example, much data is accumulating on the epidemiology of certain parasitic diseases and on the physiological properties of certain animal parasites. Your colleague in physics has an analog computer. You are in the position to program your information and predict from the computer some new attributes of a Parasitism involving Host A and Parasite Y. You are furnished today with a bulk of information on the induction of certain physiological properties of microorganisms, e.g., altered permeability. Tomorrow, you will use this information to *induce* enhanced susceptibility to a drug or to induce a suicidal physiological state.

You are living in an age when parasitism and symbiosis may become important in the exploration of space. You may have obtained a strain of euglenoid flagellates which will live in the skin of hairless rodents. In the light, these euglenoids photosynthesize and satisfy a considerable fraction of the caloric requirement of these rodents. In the dark these flagellates are parasitic. You must find a way to reduce the infection to a minimum in the dark and bring it to a maximum in the light since we must use these rodents for food. Does this sound far-fetched? It isn't, young man, if Oppenheimer is correct in his estimate that 80 percent of our knowledge has been acquired since the turn of the century. You are living in a time when knowledge is accruing at breath-taking speed. Every day that passes is more exciting than the last. Drink deep! Read widely in other fields and bring your strange new knowledge to bear on problems at hand.

The hypothetical examples above involve applied parasitology. From the standpoint of new theory or generalization, it is more difficult to cite hypothesis. We can look to you, young man, to examine parasitism in quite new ways. Perhaps you may quantitate infection as it grades into infection with disease in terms of alteration in the rates of entropy change. Perhaps you may generalize in thermodynamic terms the irreversible selection for loss of certain functions with the evolution of parasites. Perhaps you may discover wide new functional parameters in "contact ecology" which will include commensalism, mutualism, and parasitism. There are unlimited opportunities if you will use your eyes, your ears, and, above all, your brain.

(*By* Dr. Clark P. Read, *Department of Biology, The Rice Institute, Houston, Texas.*)

AMERICAN SOCIETY OF PARASITOLOGISTS

Forty-Ninth Council Meeting, University Park, Pennsylvania, August 30, 1959

The meeting of the Council of the American Society of Parasitologists was called to order by President Aurel O. Foster at 2:10 P.M. on Sunday, August 30, 1959, in the Extension Conference Center, Pennsylvania State University, University Park, Pennsylvania. In addition to President Foster, the meeting was attended by President-Elect H. W. Brown, Past Presidents J. E. Ackert, G. F. Otto, E. W. Price, B. Schwartz, and A. C. Walton, Treasurer M. J. Ulmer, the Secretary, Editor E. R. Becker, Custodian W. S. Bailey, Councilors G. H. Ball, R V. Bangham, P. C. Beaver, F. J. Kruidenier, D. V. Moore, L. J. Olivier, D. A. Porter, and M. Voge (non-voting), and by Committee Representatives or Delegates J. S. Andrews, M. Doss, K. C. Kates, H. W. Manter, A. McIntosh, N. D. Levine, D. R. Lincicome, and C. P. Read.

I. Reports of Officers and Members of the Council

1. *President (A. O. Foster)*: No report beyond the President's letter of July 15, 1959, that had been distributed to all concerned by mail.

2. *President-Elect (H. W. Brown)*: No report.

3. *Vice-President (R. M. Stabler)*: No report.

4. *Secretary (P. E. Thompson)*: As of August 25, 1959, the Secretary's records show that there are 1102 names on the current rolls of the Society. The distribution of these is as follows: 965 are Active Members in good standing, 8 are non-subscribing Inactive Members, 47 are delinquent as of this date for 1959 dues, 31 are delinquent for both 1958 and 1959 dues, 33 appear to be delinquent for 1957–1959 dues and will be dropped if their records are confirmed by the Treasurer, 8 are Emeritus Members, and 13 are Honorary Members (3 of these also are Active Members). Since the last meeting, notices of the deaths of 3 members have been received, 2 members have resigned, and one was dropped as of January 1, 1959, for non-payment of dues. During the period of this report, 56 Active Members, one Emeritus Member, and one Honorary Member have been elected to the Society. Later in this meeting, the names of 7 applicants will be submitted as candidates for active membership.

The report was accepted and ordered placed on file.

5. *Treasurer (M. J. Ulmer)*: The report was submitted in three parts owing to the change in the treasurership during the interim since the last meeting.

a. The checking account report submitted for Retiring Treasurer R. M. Stabler for the period August 1, 1958, to November 18, 1958, is summarized as follows:

Balance on Hand, August 1, 1958	$3195.55
Collections to November 18, 1958	2675.89
National Science Foundation Grant	3410.00
Total	$9281.44
Disbursements Through November 18, 1958	3846.73
Balance on Hand, November 18, 1958	$5434.71
Check Forwarded to Treasurer M. J. Ulmer	5434.71
Balance in Hands of Retiring Treasurer, R. M. Stabler	0.00

b. Treasurer M. J. Ulmer's report on regular funds for the period November 18, 1958 to August 1, 1959 is as follows: An increase in the number of members of the Society (now 965) and in the number of subscriptions sold (1110) has necessitated an increase in our print order from 2500 to 2600 copies of the *Journal*. Despite increased printing costs this year, the recent advance in subscription rates and in dues should enable us to make some progress financially. Not yet paid from our current assets are Nos. 3 and 4 and the Supplement of the current volume.

In summary:

Balance on Hand November 18, 1958	
Checking Account	$ 5434.71
Savings Account	999.92
	$ 6434.63
Collections, November 18 '58–Aug. 1 '59	17721.96
Total	$24156.59
Expenditures, November 18 '58–Aug. 1 '59	15461.61
Total Cash Balance August 1, 1959	$ 8694.98

c. Treasurer M. J. Ulmer's report on the Endowment Fund for the period August 15, 1958–August 1, 1959 is summarized as follows:

	1958	Interest Earned	1959
Savings Account*	$ 505.65	$ 9.61	$ 515.26
U.S. Savings Bonds**	887.00	27.60	914.00
	$1392.65	$36.61	$1429.26

Subject to audit by the Auditing Committee, the reports were accepted and ordered placed on file.

6. *Custodian (W. S. Bailey)*: The report submitted for the period August 1, 1958–August 1, 1959 is as follows:

Balance on Hand, August 1, 1958	$5,648.58
Net Collections During Period	6,127.93
Expenditures During Period	4,202.49
Balance on Hand, August 1, 1959	$7,574.02

Includes National Science Foundation Grant of $3410.00 for Journal of Parasitology Index, and interest of $135.47 earned by these funds.

Subject to Audit by the Auditing Committee the report was accepted and ordered placed on file.

7. *Editor (E. R. Becker)*: The number of manuscripts received for publication from January 1 to August 21, 1959, totaled 147, including research notes. Of these, 97 had been processed and 50 were either being reviewed or had been returned to the authors for more or less revision. Eighty-one of those processed were accepted for publication, 14 were rejected, and 2 were withdrawn after request for revision. Nineteen of the manuscripts accepted were published, while 62 await their turn.

Sixty-one regular papers and 29 research notes have been published in nos. 1 to 4 of vol. 45, 1959. Inspection of the subject matter reveals that 35 of the regular papers (57.4 percent) dealt with helminths, 15 (24.6 percent) with protozoa, 10 (16.4 percent) with arthropods, and 1 (1.6 percent) with both helminths and protozoa.

No. 6 of vol. 44 and nos. 1, 2, and 3 of vol. 45 cost the Society $2,491.67, $2,552.87, $2,072.36, and $2,355.34, respectively. The cost per page for the 4 numbers was, respectively, $20.76, $19.80, $18.50, and $21.03. The printer increased his charges 5 percent early in the year and has served notice of another 5 percent increase in December. The Society is faced with the critical problem of financing the Journal of Parasitology for the next few years.

The report was accepted and ordered placed on file with a vote of appreciation to Arizona State University for generous services and assistance to the Journal.

II. Reports of Standing Committees

1. *Committee on Visual Education (M. S. Ferguson, Chr., E. K. Markell, D. V. Moore, J. C. Swartzwelder, M. Voge)*: No report.

2. *Committee on Nomenclature and Terminology (H. W. Manter, Chr., G. W. Hunter, III, F. J. Kruidenier, L. R. Penner, G. W. Wharton, A. McIntosh, Advisor, N. R. Stoll, Consultant)*: Finalizing of the text of the London Code, including Glossary and Appendices, is in its late stages, prior to its being submitted to the Commission for approval. The Editorial Committee, with members from three countries, and under the requirement of the Congress to produce texts of equivalent meaning in English and French, has found its job no easy one. The Committee members and Mr. Melville have all worked with such unremitting assiduity and good spirit as to commend admiration. It is predicted that the first comprehensive official Code since the Regles adopted in 1901 by the Berlin Congress a full 58 years ago will be widely commended by the zoologists of the world.

This report, which was prepared by Dr. Norman Stoll but was received too late for the Council Meeting, was read by Chairman Manter during the Thirty-Fourth Annual General Meeting. No action was taken on the report by the Council.

3. *In Memoriam Committee (J. E. Ackert, Chr., W. S. Bailey, S. Chernin, H. W. Stunk-*

* No. 1705X of the Ames Building and Loan Association, Ames, Iowa in the name of "American Society of Parasitologists".

** No. M1,681,662F of $1000.00 in the name of "American Society of Parasitologists, a corporation (Endowment Fund)" issued January, 1950. Purchase Price $740.00.

ard) : The Committee reported that the following members of the Society have passed away since the last meeting of the Council: Asa C. Chandler, Howard E. Enders, and Herbert C. Johnstone.

The report was accepted with a deep sense of loss to the Society and ordered placed on file.

The Council also decided that for this year and henceforth it would be appropriate for the *In Memoriam Committee,* in addition to its traditional function of reporting on deceased members, to recommend to the Editor regarding obituaries for publication in the Journal and to suggest who might prepare them.

4. *Public Relations Committee* (*G. F. Otto, Chr., J. M. Andrews, A. C. Cuckler, B. Schwartz, W. H. Wright, H. W. Brown, Advisor*) : The Committee recommended that the Council advise both the State Department and the A.I.B.S. that we are actively interested in an exchange program of Russian-American parasitologists, but that it would be practical only if the A.I.B.S. can assume the scheduling and legal responsibilities, with the A.S.P. actively participating.

In reply to a request by the American Society of Ichthyologists and Herpetologists that the A.S.P. endorse its Resolution opposing the Fire Ant Control Program, the Committee recommended that the sponsors be advised that while in complete sympathy with the principal aims and objectives of its Resolution the A.S.P. cannot support the Resolution as drafted. It was further proposed that the A.S.P. urge continuing research, support whenever and wherever possible such research, and while not condoning unnecessary destruction of wildlife, assure the U.S.D.A. of our confidence and support in the continuing effort to obtain scientific data on control procedures.

The Committee recommended that no action be taken on the requests for Society support of H.R. 2165, A Bill To Establish A National Grammar Commission, and of H.R. 11018, A Bill To Increase The Income Tax Exemption For Children in College.

Chairman G. F. Otto reported that he had written letters in behalf of the Society in support of (a) the budget for the National Institutes of Health, and (b) a proposed increase of $100,000 for the Gorgas Memorial Institute.

In response to a request by the editorial staff of *Health* (published by the American Osteopathic Association) for source material including illustrations on "Parasitology As A Career", the Committee recommended that we supply a copy of the forthcoming Career Booklet (that is now under preparation for general distribution by the Career Booklet Committee) to the editor of *Health* along with the statement that the Public Relations Committee would like to have an opportunity to review and criticize any resulting article before it is published.

No member of the Committee found it convenient to attend the International Congress on Scientific Information (Washington, D. C., November 16–21, 1958).

The Committee plans to give further thought to the scope of its activity and to the modus operandi.

The report was accepted and ordered placed on file, and the Chairman was asked to write the necessary letters that stem from the foregoing report.

5. *Awards Committee* (*P. C. Beaver, Chr., F. C. Bishopp, N. D. Levine, E. W. Price, L. A. Stauber*) : The Committee reported that Clark P. Read had been unanimously selected as the 1959 recipient of the Henry Baldwin Ward Medal.

6. *Tellers Committee* (*M. F. Jones, Chr., R. E. Thorson, M. M. Farr*) : It was reported that the Committee had discharged its function of tabulating the results from the ballots returned in response to the annual call to the entire membership of the Society for suggestions regarding the various elective offices and of transmitting these results to the Chairman of the Nominations Committee.

The report was ordered placed on file.

7. *Committee on Honorary and Emeritus Members* (*L. J. Olivier, Chr., G. H. Ball, L. Jackowski, I. G. Kagan*) : The Committee, having ascertained that vacancies existed in accord with the By-Laws, proposed that the Council recommend to the Society the election of Academician Evgenii Nikonorovich Pavlovskii as an Honorary Member and Dr. Ernest Carroll Faust as an Emeritus Member.

The recommendations of the Committee were approved, and the report was ordered placed on file.

8. *Nominating Committee* (*J. S. Andrews, Chr., G. H. Ball, R. V. Bangham, P. C. Beaver, F. J. Kruidenier, D. V. Moore, L. J. Olivier, D. A. Porter, P. P. Weinstein*) : Chairman J. S. Andrews reported that the Committee would be prepared to submit for Society action during the Annual Business Meeting a slate of nominees for the elective offices of president-elect, vice-president, secretary, and two council members-at-large. In accordance with the By-Laws a Sub-Committee (C. G. Goodchild, Chr., R. M. Lewert, D. V. Moore) had served in the special capacity of proposing to the Committee a nominee for the position of secretary.

The Committee suggested that the Council empower the President to appoint a committee to

study the method of electing officers and to make recommendations for future Council consideration.

The suggestion was adopted by the Council.

III. Reports of Representatives and Delegates

1. *To Council of the A.A.A.S.* (*J. M. Andrews, G. W. Wharton, Alternate*) : J. M. Andrews submitted a six-page report by mail but it contained no issues of sufficient special concern to the Society to warrant entry in these minutes.

The report was accepted and ordered placed on file.

2. *To Governing Board of the A.I.B.S.* (*A. C. Walton, L. J. Olivier, Alternate*) : The work session of the Governing Board was held at the headquarters of the A.A.A.S. on April 28 and 29, 1959. Of major interest to our members are the following reports:

(A) Dr. Cox reported that the deficit faced at the end of the 1958 fiscal year had been entirely wiped out and a small surplus set up, and that the prospects for the current year were for some small addition to the surplus. This sound basis is due to the enlarged activities of the Institute in handling research grants and contracts, and also to enlarged membership in terms of more member societies in the Institute.

(B) Meeting places for the future were announced as follows: 1960, Oklahoma State University; 1961, a choice between Purdue, Michigan or Illinois; 1962, Univ. of Colorado or Oregon State College (depending upon building programs being completed) ; 1963, Univ. of Massachusetts.

(C) The Civil Service Commission is to reclassify biologists in government service during July, 1959, to bring their status in line with that of other scientists. Meanwhile as a temporary measure biologists may enter governmental service at a grade level as much as two steps above current practice, with permanent status to await the reclassification results.

(D) Mr. Harwood has joined the A.I.B.S. as Director of all A.I.B.S. publishing activities, both present and projected. The two journals now published are currently solvent and the 6 Russian journals now being translated have gained a growing list of subscribers. The possibility of translations of some Chinese journals is now under study and suggestions as to possible selections will be welcomed. A tentative study seems to indicate that if a number of society journals should make use of a central publishing office, expenses would run somewhat higher than they do at present for such journals as now have the benefit of free editorial services, but would be less than such journals would face if forced to employ paid editorial assistance. The tentative schedule indicates a cost of about $1.25 per member per year, plus $1.75 per page per issue as editorial costs, plus printing costs. Such figures may be of help in the survey now underway by the various individual societies.

(E) Educational projects sponsored by A.I.B.S. include: (a) 3 groups of speakers for 1959–60; one at college level involving some 80 addresses on biological problems; one on medical education and national defense involving 23 addresses at 14 different medical schools; and one on possible speakers to be available for addresses to those at the high school level; (b) the possibility of sponsoring lecture tours in this country for as many as 10 outstanding foreign biologists who might be otherwise unable to visit our universities and colleges is under consideration; (c) the setting up of a Committee on Biology Curricula for all levels, with headquarters at the University of Colorado. Already programs for teaching films at both high school and college level have been set up and some films will become available this fall (college level films on *Meiosis* and on *Speciation* are nearing completion) ; (d) the preparation of booklets for high school biology teachers emphasizing Laboratory Techniques and also Subject Matter Backgrounds advisable in the preparation of such teachers, covering those who are now "in service" as well as those yet to face their first classes.

(F) The matter of some sort of A.I.B.S. prize to be awarded outstanding biologists has been investigated and a sponsor has been located who is willing to give a very sizable sum of money and a commemorative medal to the selectee. Such an award would be offered every other year. Details as to qualifications, methods of selection of the recipient, methods of presentation of the award, and various other matters are now being worked out by the Executive Committee and the donor of the award.

(G) A "Public Relations" program is undergoing thorough study.

The report was accepted and ordered placed on file.

3. *To Division of Biology and Agriculture, National Academy of Sciences-National Research Council* (*N. D. Levine, W. S. Bailey, Alternate*) : The annual meeting of the NAS-NRC was held March 19–21, 1959, at Washington, D. C.

During the annual meeting of the Division (H. Burr Steinbach, Chr.) brief reports were given by the chairman of the Agricultural Board, Agricultural Research Institute, Food and Nutrition Board, Food Protection Committee and Institute of Laboratory Animal Resources. In

the last, Chairman Dale W. Jenkins stated that about 35,000,000 laboratory mammals were produced last year, half by 160 commercial producers and the other half by hospitals, federal and other research agencies, etc. Standards for laboratory mice and rats have been established, and it is planned to establish standards for guinea pigs. A Committee on Cell and Tissue Cultures has been established. Its major problem was felt to be the establishment of a cell registry. The problem of facilities for biological research (buildings, herbaria, museum collections, etc.) was discussed. Dr. Steinbach said that he would like to establish or re-establish the following three general programs in the Division: (a) renewable natural resources, (b) biological education, with particular attention to the production of scholars in the biological sciences, (c) agricultural policy as it affects and is affected by agricultural research.

A new catalog of NAS-NRC publications is available, and the NAS-NRC is now equipped to display at society meetings publications thought to be of interest to the society or societies concerned. Several society representatives asked how they could bring more to their societies from the NAS-NRC.

Major items discussed during the Annual Meeting of the NAS-NRC included the following subjects: (1) *The nature and conduct of fellowship programs in science.* The need for more interdepartmental and interdisciplinary cooperation and coordination was emphasized. (2) *U.S. Science in foreign aid.* The support of many small projects versus the support of one or a very few large ones was discussed. The need to develop a scientific "foreign legion" core of scientists was endorsed and means for implementing this idea were discussed. (3) *Means and patterns for the support of scientific research.* Better ways are needed to make scientific advice available to Congress. Now that adequate funds are available the problem is to use these funds to the best advantage, *e.g.,* to provide sustained support for more basic research. Possible approaches and barriers toward this goal were discussed. (4) *A major survey of needs in tropical medicine is being conducted by the Division of Medical Sciences.* This is under the direction of W. H. Wright and, among other phases, will include many items of particular interest to parasitologists.

In addition to participating in the above meeting, the ASP Representative was asked during the year for suggestions regarding the Division's activities. He recommended establishment of a Sub-Committee on Wildlife Diseases and Parasites within the proposed Committee on Renewable Natural Resources, suggested the preparation of a dictionary of biological terms, suggested a world wide cooperative intensive study of the Cervidae or at least of their diseases and parasites, and urged that the NAS-NRC support completion of the Index-Catalogue of Medical and Veterinary Zoology if the opportunity arose.

The report was accepted and ordered placed on file.

4. *To Agricultural Research Institute of the National Research Council* (*C. M. Herman*): "The last meeting of the Agricultural Research Institute was held October 13–14, 1958. As representative of the Society, I attended all sessions. Nothing of particular interest in parasitology came up for discussion.

"At the meeting in the fall of 1957 me presented three proposals relative to parasitology problems for the consideration of the ARI. To date we have had no word of any consideration or attention to these proposals. Immediately following submission of these proposals, vacancies from deaths and resignations of the key personnel of the Committee on Animal Health delayed action on many proposals. This committee has been reorganized and reported active during the past year. Therefore, it is anticipated more can be learned of the disposal of our proposals at the next meeting of the ARI in October, 1959."

The report was accepted and ordered placed on file.

5. *To National Council on Trichinosis* (*W. H. Wright*): No report.

Owing to the infrequency of the meeting of this group and the expiration of the representative's term of office, the Council decided that re-appointment would not be made in this capacity until a need arises for a representative.

6. *On American Board of Microbiology* (*N. D. Levine*): The American Board of Microbiology was established May 11, 1959, by the American Academy of Microbiology to set up and maintain a certification program for microbiologists. It is to be composed of 12 persons, of whom 5 are Fellows of the Academy and the other 7 are nominated by selected scientific societies. In addition to these, the American Medical Association has agreed to nominate one member but has not done so yet.

The Board will hold its first meeting in the fall of 1959. At that time, standards and examination committees will be established to explore the desirability of initiating clinical and public health laboratory certification programs in the following fields: bacteriology, virology, parasitology, mycology, and immunoserology.

The report was accepted and ordered placed on file, but in doing so the Council expressed little enthusiasm for the certification program plans disclosed in the report.

7. *Of Southern California Parasitologists* (*M. Voge*): During the past academic year, nine

regular meetings were held. Various members of the group and invited guest speakers (including Dr. P. J. Deoras of the Haffkine Institute, Bombay, India, and Dr. Bruce Newton of the Medical Research Council, Cambridge, England) presented papers for discussion. Announcements of these meetings with the speakers and titles are appended to this report. In addition, one meeting was devoted to the discussion of the recent article by Huff et al entitled, "An approach toward a course in the principles of parasitism."

Dr. Yost Amrein of Pomona College was elected president of the Southern California Parasitologists for the year 1958–1959. Dr. Marietta Voge was selected to serve as the non-voting SCP representative for the Forty-ninth Council Meeting at Pennsylvania State College, August, 1959.

During the year it was announced to the members that the American Society of Parasitologists and The American Society of Tropical Medicine and Hygiene had agreed to hold their joint meeting in Los Angeles in 1960. The president of the Southern California Parasitologists will appoint committees to assist with various details and local arrangements.

8. *Of Annual Midwestern Conference of Parasitologists* (*F. J. Kruidenier*): The eleventh Annual Midwestern Conference of Parasitologists, Dr. G. F. Otto, Presiding Officer, was held on the campus of Northwestern University, Evanston, Illinois on June 15–16, 1959. Eighty members attended and 14 demonstrations were presented.

Dr. Henry Van der Schalie, Museum of Zoology, University of Michigan presented the annual address. His topic was "Contrasting Problems in the Control of Schistosomiasis in Egypt and in the Sudan."

A floor discussion of the use of radioisotopes in parasitology was led by Dr. S. E. Knapp, Department of Veterinary Science, Oregon State College following a moving picture explaining isotope activity. A demonstration of isotope instrumentation was presented by several commercial concerns.

The members of the Conference were shown the facilities in North Chicago of the Scientific Division of the Abbott Laboratories by Dr. G. F. Otto, Head, Parasitology Section.

The Conference selected Purdue University, Lafayette, Indiana as the site of its 1960 meeting, taking advantage of the Symposium on Growth scheduled there for June, 1960. It voted to hold its 1961 meeting at Ohio State University, Columbus, Ohio.

Dr. F. J. Kruidenier was elected Presiding Officer for 1959–60, and Dr. D. T. Clark was elected Secretary-Treasurer for 1959–1961.

The report was accepted and ordered placed on file. (In a separate report to the Secretary Dr. Kruidenier supplied a list of 25 members of the Annual Midwestern Conference of Parasitologists that are active members of the ASP in 1959.)

9. *Inauguration of Mason Gross as President of Rutgers University* (*C. P. Read*): The Society was invited to send a representative to participate in this ceremony, and the Representative attended and conveyed the best wishes of the Society to the Rutgers University and to Dr. Gross.

The report was accepted with thanks to Dr. Read for traveling to this meeting at his own expense and was ordered placed on file.

IV. Reports of Special Committees

1. *Annual Meetings Committee* (*N. D. Levine, Chr., D. P. Furman, C. M. Herman, G. W. Hunter, III, D. L. Price*): The Committee submitted the following recommendations: (a) *Regular Sessions.* That timing device be used to prevent speakers from exceeding their allotted time; that speakers be asked not to read their speeches but to talk informally to their audience; that suggestions for preparing effective lantern slides for scientific meetings be sent to the members along with the call for papers. (b) *Demonstration Session.* That an effort should be made to enlarge our demonstration sessions. The regular demonstrations of new material should be continued but in addition some special subject of more or less general interest should be selected each year or every other year and the members be urged to bring demonstrations of old or new material dealing with it. (c) *Informal Discussion Session.* That a session be arranged which would have a moderator but no formally designated speakers. The subject would be announced beforehand and a general invitation issued to everyone to take part from the floor. (d) *Regular Symposia.* That if formal symposia are held in the future, the speakers should be reminded that they are talking to an audience which contains many people with relatively little knowledge of their subject and that their oral presentations at least should be designed to attract and hold the interest of these people. In planning each symposium an attempt should be made to organize it into a coordinated whole. (e) *Refresher Course.* That the American Society of Parasitologists suggest to the American Society of Zoologists that the two societies collaborate in putting on a refresher course on recent advances in parasitology. (f) *Luncheon-Business Meeting.* That a questionnaire be sent to the members of the Society asking for their preferences on these items. (g) *Prepublication of Abstracts.* That the desires of the members in this matter

be ascertained by means of a questionnaire before a final decision is made as to whether to discontinue publication of abstracts of papers presented at the meeting. (h) *Scheduling of Sessions.* That the members be asked to indicate their order of preference concerning the following measures in order to help the Secrtary in planning the program: Institute a screening system so that all papers submitted for presentation are not accepted, schedule simultaneous sessions, schedule one or more evening sessions, and schedule papers on an additional day or half day. (i) *Meetings.* That the Society arrange a separate meeting at the earliest open year and that a decision be made afterwards whether such meetings should be held periodically thereafter. (j) *Program Officer.* That the office of Program Officer be established in the ASP, that his term be 2 years, and that this term be staggered with that of the Secretary. This would necessitate a change in the By-Laws. Pending this change, the Program Officer could be appointed rather than elected. (k) *Further Action.* The draft of a questionnaire to be sent to the membership in order to obtain their views on the matters set forth above is appended to this report. It is recommended that the present Committee be retained on an ad hoc basis for another year to analyze the returns on the questionnaire and to make recommendations based on it at the next Council meeting.

The Council requested that the report be placed on file as an interim report of the Committee, but not necessarily accepted by the Council, and that the Committee continue its work along the lines of sending the proposed questionnaires to the membership.

2. *Business Operation Committee* (*L. Jacobs, Chr., G. F. Otto, A. C. Walton*): No report was given owing to the Chairman Jacobs unavoidable absence from the Council Meeting.

3. *Career Booklet Committee* (*D. V. Moore, Chr., R. A. Cable, D. L. DeGuisti, G. L. Hoffman, C. G. Goodchild, D. A. Porter, R. E. Thorson*): The Career Booklet Committee has prepared a draft of a proposed booklet on career opportunities in parasitology for use by high school vocational guidance personnel and high school students The present draft of the booklet needs considerable revision before it can be presented to Council for approval and further action.

Within the next few months the Committee should be able to complete the revision and begin to consider illustrations which will make the booklet more attractive. As soon as the Committee is satisfied with the results of its labor a copy of the proposed booklet will be mailed to officers and council members along with suggestions for further action by the Council.

The Secretary of the ASP has continued to refer all requests for information on Careers in Parasitology to the Chairman. The number of requests received each year indicates that this projct should be completed. It is therefore recommended that Council continue the Career Booklet Committee on an ad hoc basis with no change in personnel except for an additional committee member to assist with illustrations.

The report was accepted and ordered placed on file.

4. *Journal Index Committee* (*W. S. Bailey, Chr., M. A. Doss*): The Committee reported that the preparation of Index for Volumes 26–44 of the Journal is progressing at a commendable rate and recommended that the current volume (45) also be indexed at this time. The Committee predicted that printing costs of the Index will almost certainly exceed the cost projected a year ago when it was anticipated that $6,420 would cover the expenses of both preparation and printing. The Committee proposed two methods of raising the additional needed funds, namely, to initiate a pre-publication plan of sale at a reduced rate and to borrow from available operating funds in the Custodian's Office at the discretion of the Custodian.

The report was accepted and ordered placed on file, and the Council authorized the Committee to proceed along the lines of its recommendations.

5. *Medallion and Seal Committee* (*J. F. Mueller, Chr.*): Dr. Mueller reviewed the background and his interpretation of the Society Seal, which the Council had adopted by mail in reply to a communication from the Secretary on August 10, 1959. No report was given during the Meeting on the design and fabrication of the Henry Baldwin Ward Medal, as this phase of the Committee's work is still in the planning stage.

The report was accepted with a special vote of appreciation to Dr. Mueller for his services in designing the Seal. It also was agreed that it would be appropriate for Dr. Mueller to present and interpret the Seal to the Society during its 34th Annual General Meeting.

6. *Public Health Laboratory Problems Committee* (*M. M. Brooke, Chr.*): In a letter of February 16, 1959, to the President of the American Society of Parasitologists, Dr. M. Scherago stated: "The Committee (on Public Health Laboratory Problems) has been continued for the current year. As soon as I find that our plans for the year have been definitely formulated, I shall communicate with Dr. Brooke." To date no communication has been received from Dr. Scherago so the ad hoc Committee has been inactive. Since the diagnosis of parasitic diseases constitutes a problem to many public health laboratories, a liaison with the APHA Committee should be desirable. The ultimate value and potentialities of such an association depends at present on initiative of the parent Committee."

The report was accepted and ordered placed on file.

7. *Russian Translations Committee* (*K. C. Kates, G. Anastos, E. C. Dougherty, M. A. Doss, L. Jacobs*) : The Committee reviewed the cooperative efforts of several members of the Society with the A.I.B.S. translations program, which includes plans for several publications in parasitology. It reported that 4 Russian monographs in parasitology are scheduled for translation under the auspices of the National Science Foundation. A revised copy of important Russian publications in parasitology by Miss Mildred Doss was presented, and plans to distribute copies to the membership during the demonstration session were announced. A list was submitted of the titles of 172 abstracts in The Proceedings of the Scientific Conference of the All-Union Society of Helminthologists Celebrating the 40th Anniversary of the Great October Socialist Revolution, 11–15 December, 1957. The Committee supplied information requested by the NSF Survey Committee on Russian-English Lexicography. The Committee recommended that the Society continue to cooperate with all individuals and agencies interested in providing translations of important foreign parasitological publications, and urge its members to inform appropriate Society officials of the availability of new translations of important publications in parasitology, so that steps can be taken to make them available to other members of the Society.

The report was accepted and ordered placed on file, and it was voted that the Russian Translations Committee be continued.

8. *Auditing Committee* (*M. Voge, Chr., R. V. Bangham, L. Olivier*) : The Committee has examined the Treasurer's and the Custodian's reports of the American Society of Parasitologists for the year 1958–59 and have found them to be in order.

Recommendations for the future: (1) That a summarization of all assets and liabilities of the American Society of Parasitologists, including the value of equipment and back issues, accompany the treasurer's report. (2) That the fiscal year of the society be the same each year, regardless of the date of the annual meeting. (3) That the books of the American Society of Parasitologists be audited by a certified public accountant at the end of the terms of custodian or treasurer, and that the Council consider the desirability of more frequent audits.

The report was accepted and ordered placed on file.

V. Old Business

1. *Henry Baldwin Ward Medal Award:* Chairman P. C. Beaver presented a series of proposals directed toward clarification of the qualifications of the recipient, qualifications of the nominators, modus operandi, and deadline for nominations. Most aspects of these proposals had appeared in the 1959 Call for Nominees and they differed slightly from the Minutes of the 1958 Council Meeting. The proposals finally submitted for action during the 1959 Council Meeting were as follows:

In honor of the Society's first president and founder of its *Journal of Parasitology*, the award shall be designated *The Henry Baldwin Ward Medal.*

The recipient shall be a member of the Society who by 15 years or less of self-directed investigations will have attained a position of leadership in some phase or phases of parasitological research, the term parasitology being interpreted as including, in general, those fields represented by reports published in the official organ of the Society, The Journal of Parasitology.

Subject to Council ratification selection of recipients shall be made by an Awards Committee consisting of five members representing the several disciplines within the Society, appointed by the President for two-year terms, except for two of the first appointees who shall serve one year. Members of the Council shall be ineligible for consecutive appointment.

Nominations of recipients shall be made by members of the Society through the Secretary and must be received six months prior to the next scheduled annual meeting at which time the Award ordinarily will be presented.

An Awards Committee may declare "no award" if unable to recommend a recipient three months prior to the scheduled date of presentation.

The Award shall be presented in the name of the Society at the annual general session, and it shall be deemed appropriate for the recipient to present a review of his work at that time.

The foregoing proposals were ratified by the Council, and the report was accepted and ordered placed on file.

VI. New Business

1. *Ratification of the A.I.B.S. Articles of Incorporation (Article 3) to Permit the A.I.B.S. to Qualify as a Tax-Exempt Organization.* The Council, having been duly informed of the nature of the above changes, acted in behalf of the Society in ratifying these changes and instructed the Secretary to advise the A.I.B.S. accordingly.

2. *Request of the A.I.B.S. Central Publications Office for a Gratis Subscription to the Journal of Parasitology.* Consistent with the practice of most member societies of the A.I.B.S., the Council voted that the A.I.B.S. Central Publications Office be placed without charge on the mailing list for current and future issues of the Journal. The Treasurer was instructed

to send all issues of Volume 45 and to send future issues until the Council directed otherwise.

3. *Application of the Helminthological Society of Washington for Affiliation with the American Society of Parasitologists.* A duly executed application had been filed with the Secretary on February 2, 1959, except that the applicant stated its inability to meet the A.S.P. By-Laws (Article VI, Section 4) requirement that all of its officers be members of the A.S.P. It was explained to the Council (by D. R. Lincicome, Recording Secretary of "Helmsoc," and others) that this ability stemmed from the fact that a considerable number of the "Helmsoc" members are not members of the A.S.P. and that compliance with the A.S.P. By-Laws would make such people ineligible for office in their own Society.

The Council voted its acceptance of the application by the Helminthological Society of Washington as an affiliate of the American Society of Parasitologists subject to Society amendment of the A.S.P. By-Laws. The Secretary was instructed to inform "Helmsoc" accordingly.

4. *Amendments of By-Laws.* The Council voted to ask for Society action on the following proposed amendments of the By-Laws.

Article VI. Regional Branches and Affiliated Organizations.

Section 4. *Officers.* A list of officers shall be filed with the Secretary within 3 weeks after their election.

Article V. Finances.

Section 5. *Endowment Fund.* The purpose of the Endowment Fund is to underwrite special projects of long-term value and interest to the Society. This purpose shall be construed conservatively and the integrity of the Fund maintained. To enlarge its opportunity of service, contributions from friends of parasitology are welcomed.

The Secretary was instructed to submit by mail the proposed amendments to the membership with a request for action.

5. *Proposition That the A.S.P. Purchase a Fixed Number of Copies of Helminthological Abstracts so That A.S.P. Members May Purchase Them From the A.S.P. at a Reduced Rate.* The Council instructed the Secretary to canvass the membership regarding interest in subscribing to *Helminthological Abstracts* at a reduced rate.

6. *Election of Custodian.* It was overlooked during the regular meeting of the Council that the term of W. S. Bailey had expired.

Subsequently, by mail, the Council re-elected the incumbent to a 3-year term (1960 to 1962) as Custodian and Dr. Bailey accepted the election.

7. *Election of Assistant Editors.* On recommendation of the Editor the Council re-elected R. B. McGhee, A. McIntosh, and P. P. Weinstein to 4-year terms (1960–1963) of office.

8. *Appointment of Society Representatives and Delegates.*

(a) *Representative and Alternate on the Governing Board of the A.I.B.S.:* To be named by the President.

(b) *Representative and Alternate to the Division of Biology and Agriculture of the National Research Council:* To be named by the President.

(c) *Representative and Alternate to the A.A.A.S. Council:* To be named by the President.

(d) *Representative to the Agriculture Research Institute of the National Research Council:* To be named by the President.

(e) *Representative to the American Academy of Microbiology:* To be named by the President.

(f) President Foster appointed, with Council approval, Benjamin Schwartz to serve as the Society's official delegate to the 1st International Conference on Trichinellosis, Warsaw, September 12–13, 1960, and the appointee stated that he expected to be able to serve in this capacity.

9. *Elections to Active Membership:* The Council voted to accept the application of 7 applicants for active membership in the Society.

10. *Appointments to Standing Committees:*

(a) *Auditing:* To be named by the President.
(b) *Awards:* Two members to be appointed for 2-year terms each.
(c) *Honorary and Emeritus Members:* To be named by the President.
(d) *In Memoriam:* To be named by the President.
(e) *Nomenclature and Terminology:* To be named by the President.
(f) *Public Relations:* To be named by the President.
(g) *Resolutions:* To be named by the President.
(h) *Tellers:* To be named by the President.
(i) *Visual Education:* To be named by the President.

11. *Appointments to Special Committees:*

(a) *Annual Meetings:* Committee continued without change in membership.

(b) *Business Operations:* Committee continued without change in membership.

(c) *Career Booklet:* Committee continued without change in membership except for one other member to be added.

(d) *Journal Index:* Committee continued without change in membership.

(e) *Medallion and Seal:* J. F. Mueller, Chr., P. E. Thompson.

(f) *Method of Electing Officers:* To be named by the President.

(g) *Public Health Laboratory Problems:* Committee continued without change in membership.

(h) *Selection of Meeting Places:* To be named by the President.

(i) *Translations:* To be named by the President.

12. *Review of Annual Meetings Plans for 1960–1963:* The Council voted that: (a) the 1960 meeting will be in conjunction with the American Society of Tropical Medicine and Hygiene, in Los Angeles, on November 2–5; (b) the 1961 meeting will be with the A.I.B.S., probably at Purdue University or the University of Illinois, in late August; (c) the 1963 meeting will be in conjunction with the American Society of Tropical Medicine and Hygiene, in Chicago, on November 6–9.

The Council empowered the President to appoint a committee to suggest plans for the annual meetings in 1962, 1964, and 1965.

13. *Miscellaneous Actions by the Council:* The Committee on Business Operations was asked to review the costs of the *Journal of Parasitology* and to make recommendations to the Council regarding means of increasing the revenue for the Journal.

The Secretary was instructed to report to Professor J. F. A. Sprent that the backlog of papers, financial status of the *Journal of Parasitology*, and precedent aspects, made it unwise to consider at this time any commemorative issues of the Journal.

The Editor was empowered to ask authors to pay printing charges in excess of $200.00 for a single publication.

The Editor was authorized to spend up to $75.00 per month for office assistance.

The Treasurer was empowered to transfer the savings account fund from the Baltimore bank to the Society's checking account in an Ames, Iowa, bank.

The Editor and Treasurer were instructed that non-member foreign subscription rates should be stated as $10.25 on the inside cover of the Journal rather than $10.00 to adjust for recent increases in postal rates.

The Council voted to adjourn at 1:00 A.M., August 31, 1959.

Respectfully submitted,
/S/ Paul E. Thompson, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Fourth Annual General Business Meeting, September 2, 1959

Following the annual luncheon held in the Hetzel Union Building, Pennsylvania State University, President Aurel O. Foster presided during the awarding of the Henry Baldwin Ward Medal, the presentation and interpretation of the Society's Seal, and the General Business Meeting.

Chairman of the Awards Committee Paul C. Beaver presented, on behalf of the Society, the first Henry Baldwin Ward Medal to Dr. Clark P. Read, and the recipeint graciously accepted the Award.

Chairman of the Seal Committee Justus F. Mueller introduced and interpreted the Seal that he had designed. The Seal had been adopted as the official emblem of the Society by the Council during August 1959, which enabled its first use as an integral part of a certificate for the recipient of the Henry Baldwin Ward Medal.

President Foster called the Business Meeting to order at 1:45 P.M.

The Secretary reported on the membership and made announcements as follows:

(1) The Society has 1109 members, of whom 971 are active and in good standing.

(2) The Helminthological Society of Washington has applied for recognition as an Affiliate of the American Society of Parasitologists, and the Council has gladly accepted the application subject to appropriate amendment of the By-Laws by the Society.

(3) Ballots will be mailed to the members of the Society in October 1959, for a vote on By-Laws amendments pertaining to Affiliated Organizations and the Endowment Fund.

(4) Future Annual Meeting plans agreed upon by the Council up to this time are as follows: 1960, November 2–5, Los Angeles, with the American Society of Tropical Medicine and Hygiene; 1961, late August and early September, Purdue University or University of Illinois with the A.I.B.S.; (1962 is under consideration); 1963, November 6–9, Chicago, with the American Society of Tropical Medicine and Hygiene.

Treasurer Martin J. Ulmer reported that owing to the increase in dues last year the Society is solvent, but that it still has little reserve funds.

Editor Elery R. Becker reported on the status of the Journal and of some outstanding papers scheduled to appear in the next few issues.

Custodian Wilfred S. Bailey reviewed the status of the Custodian's office and gave a progress report on the preparation of the Index for Volumes 26–45 of the Journal.

Chairman of the Honorary and Emeritus Members Committee Louis J. Olivier presented Council nominations as follows: As an Honorary Member, Academician Eugenii N. Pavlovskii; as an Emeritus Member, Dr. Ernest Carroll Faust. Following a call for other nominations and Society action, the above nominees were elected by the Society.

Chairman of the Nominating Committee John S. Andrews submitted the following slate of nominees for the elective offices of the Society.

President-Elect—Justin M. Andrews
Vice-President—Paul E. Thompson
Secretary (2-yr term)—Francis J. Kruidenier
Council Members At Large (4-yr terms)—Robert M. Lewert, Clark P. Read.

An invitation was made for nominations from the floor or for Society action on the nominees. The Secretary was instructed by the Society to cast one ballot in its behalf for the slate of nominees listed above.

The Society elected Lloyd E. Rozeboom as Chairman of the Nominating Committee for 1960.

Chairman of the Nomenclature and Terminology Committee Harold W. Manter read a progress report on the preparation of the London Code by the International Commission on Zoological Nomenclature that had been prepared by Norman R. Stoll. (This report appears in the Minutes of the Forty-Ninth Council Meeting).

Chairman of the Resolutions Committee Elery R. Becker submitted the following report, which was accepted by the Society.

"1. Be it resolved that the American Society of Parasitologists express its appreciation to Dr. Eric Walker, the President of Pennsylvania State University, for inviting it to hold its 34th annual meeting on this campus, and to the faculty, students and other staff members who in various ways contributed to the convenience and comfort of the participants in this meeting.

"2. Be it resolved that the American Society of Parasitologists express to Dr. T. H. Cheng its thanks for making appropriate and satisfactory arrangements for the various meetings of the Society in the lecture halls and laboratories of this campus.

"3. Be it resolved that the Society express its appreciation to Dr. R. E. Larson, Chairman of the Committee on Local Arrangements for the A.I.B.S., for the role he played in organizing the facilities on the campus for the purposes of these meetings.

"4. The Resolutions Committee moves that the American Society of Parasitologists extend to Dr. Paul E. Thompson the thanks of all its members for the years he has spent in the successful performance of his arduous duties as its Secretary. His record is one of most distinguished service.

"5. Further, the Resolutions Committee moves that the American Society of Parasitologists express its appreciation to Dr. J. F. Mueller for employing his special artistic talents in the creation of an appropriate and intriguing Seal.

"6. Further, that the Secretary be nstructed to convey to Parke, Davis and Company the gratitude of the American Society of Parasitologists for the generous gift that has made possible the annual awarding of the Henry Baldwin Ward Medal, along with generous monetary considerations, to an eminently worthy and comparatively young individual who has distinguished himself in the broad field of parasitology, and for the generous office assistance that it has extended to the Society's Secretary during his term of office."

The Society voted to adjourn at 2:20 P.M.

Respectfully submitted,
/S/ Paul E. Thompson, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS

Amendments of By-Laws of the American Society of Parasitologists

ARTICLE V. FINANCES

Section 5. *Endowment Fund.* The purpose of the Endowment Fund is to underwrite special projects of long-term value and interest to the Society. This purpose shall be construed conservatively and the integrity of the Fund maintained. To enlarge its opportunity of service, contributions from friends of parasitology are welcomed.

ARTICLE VI. REGIONAL BRANCHES AND AFFILIATED ORGANIZATIONS

Section 4. *Officers.* A list of officers shall be filed with the Secretary within 3 weeks after their election.

(*Secretary's Note:* These are the first and only amendments of the exisitng By-Laws, which were adopted by Society vote in May–June, 1957 and went into effect on January 1, 1958. A copy of the By-Laws appears in J. Parasitol. **44**: 121, 1958. These amendments, submitted to the Society by mail in accordance with Article IX of the By-Laws, were duly accepted by the Society during October–December, 1959.—PAUL E. THOMPSON. *Secretary, December 16, 1959.*)

PRELIMINARY ANNOUNCEMENT OF THE THIRTY-FIFTH ANNUAL MEETING

November 2–5, 1960, Biltmore Hotel, Los Angeles, California

The thirty-fifth annual meeting of the American Society of Parasitologists will be in conjunction with the annual meeting of the American Society of Tropical Medicine and Hygiene.

Inquiries concerning the general plans for the meeting may be addressed to the Secretary, Dr. F. J. Kruidenier (Department of Zoology and Physiology, University of Illinois, Urbana, Illinois). Dr. Gordon H. Ball (Department of Zoology, University of California, 405 Hilgard Avenue, Los Angeles 24, California) has agreed to serve as Chairman of the Society's Local Committee; the other members of the Committee are Doctors Yost U. Amrein, Donald Heyneman, Walter E. Martin, Marietta Voge, and Edward D. Wagner.

THE HENRY BALDWIN WARD MEDAL FOR 1960.

Nominations of recipients for the second Henry Baldwin Ward Medal to be awarded at the next annual meeting of the Society (Los Angeles, 2–5 November 1960) should be in the hands of the Secretary not later than the first of May.

Named in honor of the Society's first president and founder of the Journal of Parasitology, and sponsored by Parke, Davis and Company, the first Henry Baldwin Ward Medal was awarded at the thirty-fourth annual meeting in 1959 (ref.). The conditions of the award, established by the Council of the Society and stated in the minutes of the last meeting, are as follows:

The recipient shall be a member of the Society who by 15 years or less of self-directed investigations will have attained a position of leadership in some phase or phases of parasitological research, the term parasitology being interpreted as including, in general, those fields represented by reports published in the official organ of the Society, The Journal of Parasitology.

Subject to Council ratification, selection of recipients shall be made by an *Awards Committee* consisting of five members representing the several disciplines within the Society, appointed by the President for two-year terms, except for two of the first appointees who shall serve one year. Members of the Committee shall be ineligible for consecutive appointment.

Nominations of recipients shall be made by members of the Society through the Secretary and must be received six months prior to the next scheduled annual meeting at which time the Award ordinarily will be presented.

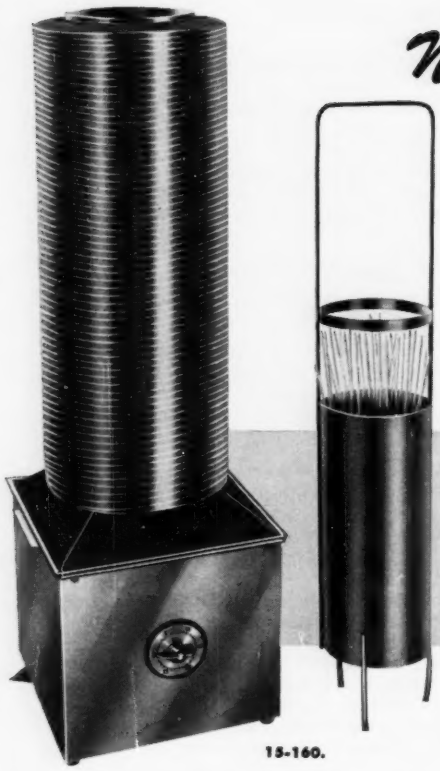
An *Awards Committee* may declare "no award" if unable to recommend a recipient three months prior to the scheduled date of presentation.

The Award shall be presented in the name of the Society at the annual general session, and it shall be deemed appropriate for the recipient to present a review of his work at that time.

Nominating letters should state fully the qualifications of the nominee including a biographical sketch, honors, publications, and a brief summary of major contributions.

New . . .

VIR TIS
FILTERED AIR
PIPETTE DRYER



15-160.

- **Dries a rack of 200 pipettes in 30 minutes or less**
- **Dust contamination from room air minimized by filter**

PIPETTE DRYER, VirTis Filtered Air Type. Combines high wattage heat, efficient insulation and high velocity convection for rapid drying of pipettes. A filter in the air stream removes contaminating dust before it enters the drying chamber. A timer switch automatically shuts off the unit at the end of a predetermined cycle.

Metal parts are of Stainless steel throughout. The 1250 watt combination air heater and blower delivers 50 cubic feet of air per minute. Drying chamber is heavily insulated with fiber glass with outer shell of heat-reflecting aluminized Mylar®; dimensions of drying chamber, 24 inches high × 5½ inches inside diameter.

A heavy duty electric timer, range 0 to 30 minutes, is built into the base. Solid wall construction of the drying rack prevents pipette breakage. Overall height of complete apparatus, 33 inches; base 10½ inches square.

15-160. Pipette Dryer, VirTis Filtered Air Type, as above described, complete with one pipette carrier and connecting cord; for 115 volts, 60 cycles, a.c.. **195.00**

15-146. Extra Pipette Carrier, 26 inches high × 5⅜ inches outside diameter; with solid Stainless steel wall and Stainless steel mesh bottom; takes 200 to 300 pipettes. **18.00**

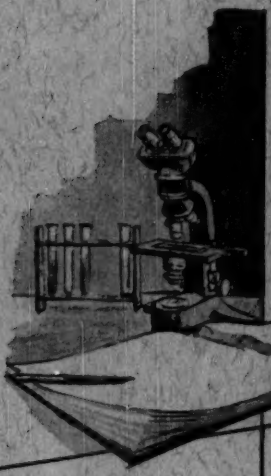
NOTE—The pipette carrier, 5¼ inches outside diameter, as supplied with the Boekel Automatic Pipette Washer, can be used in the chamber of above Dryer.

A.H.T.CO.

ARTHUR H. THOMAS COMPANY

More and more laboratories rely on Thomas / *Laboratory Apparatus and Reagents*

VINE ST. AT 3RD • PHILADELPHIA 5, PA.



notes from a MICROSCOPIST'S NOTEBOOK

FAT STAINING

Techniques and solutions for the demonstration of fat in tissue have advanced markedly since Daddi's first oil soluble dye for this purpose in 1896.

Using alcohol as the solvent for the dye, early workers often dissolved out much of the fat present. Subsequently, solutions based on equal parts of 70% alcohol and acetone were used, and more recently solutions of 50% to 60% isopropanol and ethylene glycol or propylene glycol have found favor.

Advances in solutions have been paralleled by better techniques and the availability of a wider selection of fat stains which provide greater depth of color and contrast.

National has steadily expanded its line of Biological Stains and Indicators to include the principal stains in current use for demonstrating fat in tissue.

For your convenience, we list their Catalog Numbers:

#673—Sudan II	* *#629—Sudan Black B*
* *#635—Sudan III*	*#689—Oil Red O*
* *#542—Sudan IV*	*#599—Oil Blue N*
* *#686—Nile Blue A*	

* Certified by the Biological Stain Commission

NATIONAL
BIOLOGICAL STAINS and INDICATORS

Allied Chemical

NATIONAL ANILINE DIVISION
40 RECTOR STREET, NEW YORK 6, N. Y.

CONTENTS

JOURNAL OF PARASITOLOGY

Volume 46, No. 1 February 1960

PARASITOLOGICAL SPECULATIONS AND PATTERNS. Aurel O. Foster (1– 9)

THE RESISTANCE OF THE ENCYSTED FORM OF *Toxoplasma gondii*. Leon Jacobs, Jack S. Remington and Marjorie L. Melton (11– 21)

A SURVEY OF MEAT SAMPLES FROM SWINE, CATTLE AND SHEEP FOR THE PRESENCE OF ENCYSTED *Toxoplasma*. Leon Jacobs, Jack S. Remington and Marjorie L. Melton (23– 28)

GLOBIDIUM INFECTIONS IN THE SUDAN WITH SPECIAL REFERENCE TO *Globidium gilruthi* (CHATTON, 1910) IN SHEEP AND GOATS. K. N. Soliman (29– 32)

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: AMINO ACID PERMEATION INTO TAPEWORMS FROM ELASMOBRANCHS. Clark P. Read, John E. Simmons, Jr. and Alvin H. Rothman (33– 41)

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: PERMEATION OF UREA INTO CESTODES FROM ELASMOBRANCHS. J. E. Simmons, Jr., C. P. Read and A. H. Rothman (43– 50)

PERMEATION AND MEMBRANE TRANSPORT IN ANIMAL PARASITES: THE ABSORPTION OF GLUCOSE BY *Hymenolepis diminuta*. Kenneth Phifer (51– 62)

EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE EGGS OF *Ascaridia galli*. W. Malcolm Reid (63– 67)

THE FINE STRUCTURE OF THE GROWTH REGION OF OVARY IN *Ascaris lumbricoides* VAR. *suum* WITH SPECIAL REFERENCE TO THE RACHIS. James J. Prestage (69– 78)

A CASE OF *Capillaria* INFECTION CAUSING CUTANEOUS CREEPING ERUPTION IN MAN. Kaoru Morishita and Tsuneo Tani (79– 83)

SOME HEMIURID TREMATODES OF OREGON MARINE FISHES. James E. McCauley (84– 89)

THE LIFE HISTORY OF THE SALMON-POISONING FLUKE, *Nanophyetus salmincola* (CHAPIN). Elwin Bennington and Ivan Pratt (91–100)

THE SOUTH AFRICAN TICKS *Rhipicephalus capensis* KOCH AND *R. gertrudae* N. SP. B. Feldman-Muhsam (101–108)

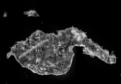
A CAGE FOR MAINTAINING STOCK COLONIES OF PARASITIC MITES AND THEIR HOSTS. Joseph H. Camin and Paul R. Ehrlich (109–111)

RESEARCH NOTES:

SOME PARASITES OF CENTRAL CALIFORNIA AMPHIBIANS: Donald L. Lehmann, 10. THE CHEMICAL COMPOSITION OF GREGARINE HARD-PARTS: Winston E. Ratnayake, 22. ON THE CONTINUOUS CULTURE OF OPALINIDS: William C. T. Yang, 32. THE ACTIVITY OF BEPHENIUM EMBONATE AGAINST THE FREE-LIVING AND ADULT STAGES OF *Nematodirus spathiger*: W. C. Marquardt, D. H. Fritts, N. R. McAlpin and W. W. Hawkins, Jr., 42. ON THE LIFE CYCLE OF *Neodiplostomum buteonis* AND THE OCCURRENCE OF A PERIPROSTATE (TREMATODA: DIPLOSTOMATIDAE): J. C. Pearson, 48. AN UNUSUAL CASE OF HUMAN POROCEPHALIASIS: Wanda Sanborn Hunter and Robert P. Higgins, 68. PEPTIDASE AND LIPASE ACTIVITY OF EXTRACTS OF *Schistosoma mansoni* CERCARIAE: S. Mandlowitz, D. Dusanic and R. M. Lewert, 89. THE EFFECT OF STILBESTROL IMPLANTS UPON MULTIPLE INFECTIONS OF *Trichostrongylus axei* AND *Trichostrongylus colubriformis* IN THE LABORATORY RABBIT: George H. Rohrbacher, Jr., 108. HUMAN AND ANIMAL SCHISTOSOMIASIS IN KHARTOUM PROVINCE, SUDAN: Emile Abdel Malek, 111.

DATES OF MAILING ISSUES OF VOLUME 45 (1959) (41)

SOCIETY BUSINESS:

THE SOCIETY SEAL, 112. THE AWARD OF THE FIRST HENRY BALDWIN WARD MEDAL, 113. THE ACCEPTANCE OF THE HENRY BALDWIN WARD MEDAL, 114. AMERICAN SOCIETY OF PARASITOLOGISTS: 47TH COUNCIL MEETING, UNIVERSITY PARK, PENNSYLVANIA, AUGUST 30, 1959, 116. 34TH ANNUAL GENERAL BUSINESS MEETING, SEPTEMBER 2, 1959, 125. AMENDMENTS OF BY-LAWS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS, 127. PRELIMINARY ANNOUNCEMENT OF THE THIRTY-FIFTH ANNUAL MEETING, 127. THE HENRY BALDWIN WARD MEDAL FOR 1960, 127.

BUSINESS PRESS INC.
Lancaster, Pennsylvania